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AMERICAN MALACOLOGICAL BULLETIN

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Cryptostrakon corcovadensis, a new species of semislug from Costa Rica (Helicoidea: Xanthonychidae) with comments on the systematic position of the genus

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Abstract: A new species of the semislug genus *Cryptostrakon* Binney, 1879, is described. A description of the external morphology as well as the pallial, reproductive, digestive, and nervous systems is carried out. Based on the new anatomical evidence the systematic position of the genus within the Xanthonychidae is reaffirmed. Comparisons among the semislug genera of the Xanthonychidae are also provided. Morphologically, *Cryptostrakon* appears to be more related to *Xanthonyx* and *Metostracon* than to any other xanthonychid genera. The phylogenetic relationships of *Cryptostrakon* are, however, still controversial and their clarification will have to wait until a testable phylogeny of the Xanthonychidae is proposed.

Key words: anatomy, systematics, taxonomy, Central America

The genus *Cryptostrakon* was described by Binney (1879) based upon a species collected by W. Gabb in Costa Rica. *C. gabbi* Binney is the type species by original designation (Binney, 1879; Baker, 1963) and until now the only component of the genus. Unfortunately, the type material upon which the species description was based is dried, as stated by Pilsbry (1900). Although Baker (1963) designated a lectotype and several paralectotypes, anatomical observations are not possible based upon them because of the poor preservation of their bodies. Consequently, the descriptions that Binney (1879) provided on the jaw, radula, shell, and external morphology of the body of *C. gabbi* remain as the only data for the genus. For this reason, until now the internal anatomy of the different systems in this genus were completely unknown.

The purpose of this study is to describe a new species of the genus *Cryptostrakon*, to compare this genus with other semislug genera, and to clarify its systematic position.

MATERIAL AND METHODS

The following material was studied (ANSP = Academy of Natural Sciences of Philadelphia; INBIO = Instituto de Biodiversidad de Costa Rica):

Cryptostracon gabbi Binney, 1879

ANSP 57992: Costa Rica (Gabb), flanks of Pico Blanco, 5000-7000 ft elevation. TYPE.

ANSP 246310, A9639: Costa Rica. Det. H. B. Baker. LECTOTYPE.

ANSP 394149: Costa Rica. PARALECTOTYPES.

Cryptostracon corcovadensis sp. nov.

INBIO 468087: Costa Rica, Madrigal, Prov. Punta, P. N. Corcovado, Estacion Sirena, Sendero a Rio Los Patos, 10 m. LS 270600_508300. 14 August 1994, Marianella Segura! HOLOTYPE.

INBIO 468080: Costa Rica, Madrigal, Prov. Punta, P. N. Corcovado, Estacion Sirena, Sendero a Rio Los Patos, 10 m. LS270600_507800. 18 August 1994. Marianella Segura!

INBIO 468059: Costa Rica, Prov. Punta, P. N. Corcovado, Estacion Sirena, Sendero Los Espaveles, 20 m. LS270600_507800. 12 August 1994. Marianella Segura!

Bunnya bernardinae Baker, 1942

ANSP A16728: ruins of monastery 20 km SW of Mexico City, El Desierto de Los Leones to La Venta, Distrito Federal, Mexico. July 1926. H. B. Baker!

Metostracon mima Pilsbry, 1900

ANSP 77245, A9636: Morelia, Michoacan, Mexico.

S. N. Rhoads! HOLOTYPE.

ANSP A9635F: Uruapam del Progreso, Michoacan, Mexico. S. N. Rhoads! PARATYPE.

ANSP A9410D: near Alvarez at km 53, San Luis de Potosi, Mexico. July 1934. H. A. Pilsbry!

ANSP A9411A: km 42, Potosi and Rio Verde Railroad, San Luis de Potosi, Mexico. August 1934. H. A. Pilsbry!

Xanthonyx sp.

ANSP A16735: 14 km from Cordoba toward Orizaba on Mexican railroad, hills southeast of town, on opposite side of Canyon Sumidero, Vera Cruz State, Mexico. June 1926. H. B. Baker!

The alcohol preserved material was dissected under a Wild M5A microscope and illustrations were made with the aid of a camera lucida. A Cambridge 200 scanning electron microscope at ANSP was used for the observation of the radula and jaws; the preparation procedure followed was as described by Ploeger & Breure (1977). Terminology for the anatomical descriptions follows Tompa (1984).

The alcohol preserved material of *Xanthonyx, Metostracon,* and *Bunnya* spp. were used for comparative proposes among the semislug xanthonychid genera. Formal descriptions on the anatomy of these taxa were not carried out; only the differences found with the original descriptions are pointed out.

RESULTS

Subclass Pulmonata
Order Stylommatophora
Superfamily Helicoidea
Family Xanthonychidae Strebel & Pfeiffer, 1879
Genus *Cryptostrakon* Binney, 1879
Cryptostrakon corcovadensis sp. nov.

Diagnostic characters

Shell depressed with spiral nucleus, entirely concealed by mantle. Visceral mass reduced with pneumostome in middle right margin. Tail mid-dorsal groove well-developed, lined with thin pigmented band. Jaw smooth, presenting only shallow vertical grooves in medial zone. Mucus glands of terminal genitalia composed of multiple thin blind sacs (tubules) enveloped by same tissue ending in one common duct inserted in medial zone of the dart sac. Atrium with thin transverse ridges in its interior.

Type locality: (see Materials and Methods).
Etymology: Named for Corcovado National Park in Costa Rica, type locality of this species.

External morphology

Semislug with visceral mass reduced and packed in medial dorsal portion of animal. Visceral mass protected by shell, completely concealed by mantle. Pneumostome wide, located in medial right margin of visceral mass (Figs. 1, 2). Openings of rectum and pneumostome clearly visible. Tail muscular with well-developed mid-dorsal groove marked by thin dorsal pigmented band that narrows, eventually disappearing toward tip of tail (Fig. 1). Foot sole not divided. Holotype 22 mm total preserved length.

Internal morphology

Shell

Shell depressed, concealed by mantle, fragile to membranous. Two whorls without septa. Aperture wide, ovate. Concentric lines of growth noticeable on last whorl. Peristome lined with thin membrane reflected toward interior of aperture. Measurements of holotype (Figs. 3, 4): Major diameter, 8 mm; minor diameter, 6.5 mm; aperture diameter, 5.5 mm; height, 4 mm.

Body cavity

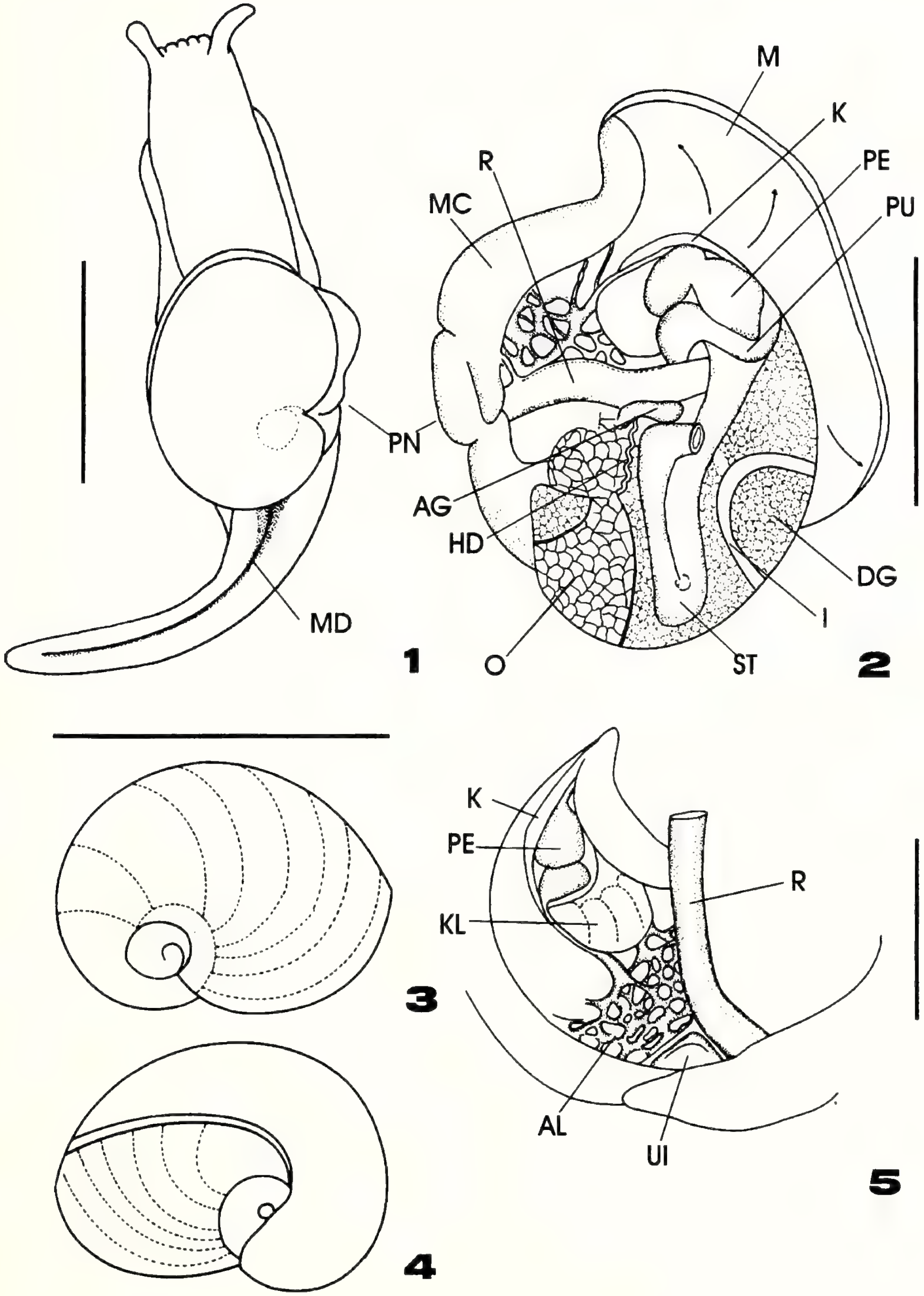
Body cavity not extending into posterior part of foot, ending immediately behind visceral mass. Anterior part of foot containing part of digestive, reproductive, and nervous systems.

Pallial system

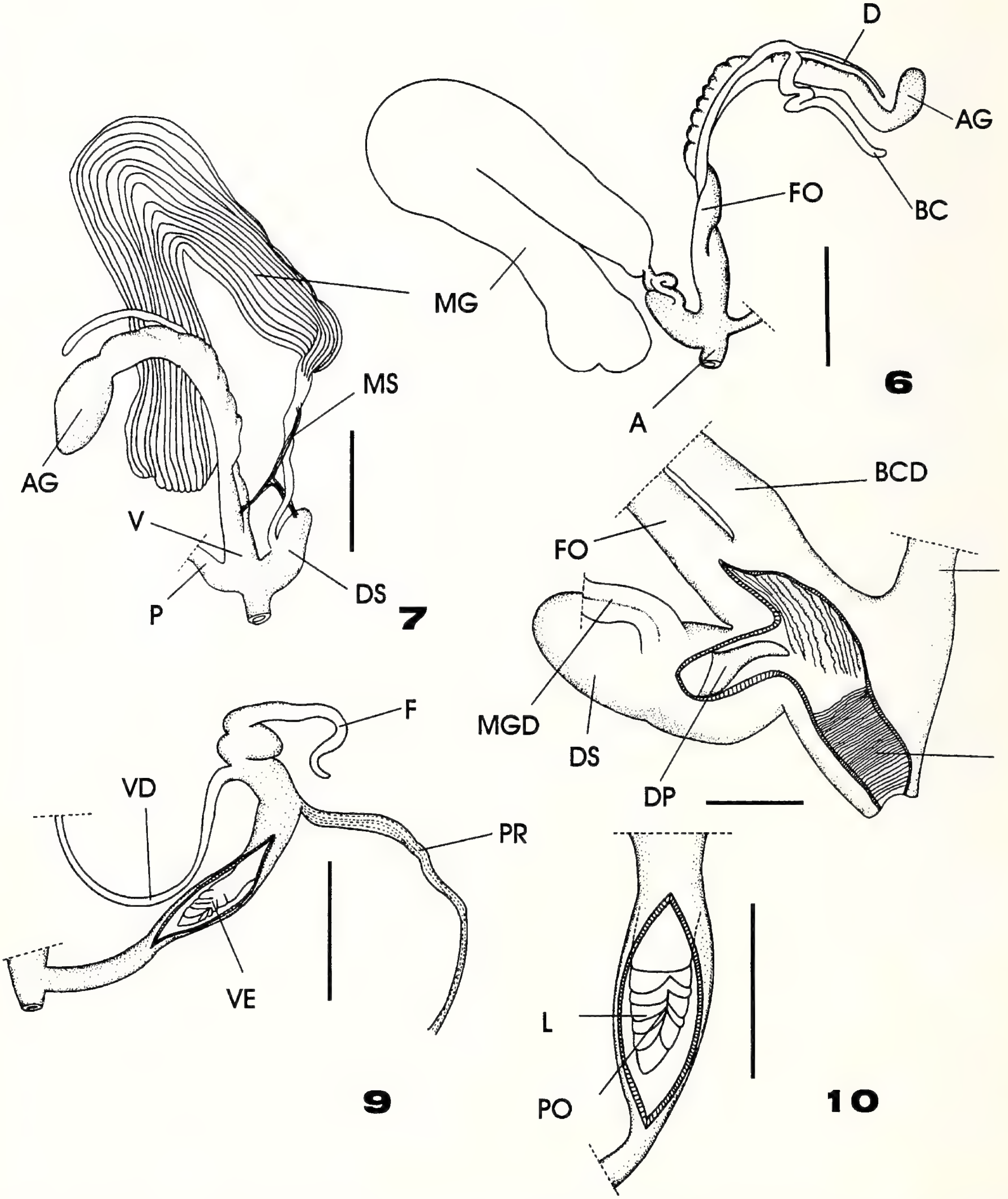
Pallial system lying under mantle and immediately beneath shell, thus protected by both shell and mantle (Fig. 2), composed of lung, kidney, ureters, and pericardium, occupying anterior left half of reduced visceral mass. Kidney, heart, and rectum perpendicular to longitudinal axis of the body. Kidney partially surrounding pericardium, with axis directed toward pneumostome. Kidney short, half the length of lung, not compact, with four inferior lobes with internal lamellae converging to kidney pore (Fig. 5). Primary ureter starting at right side of pericardium and running transversely across to rectum (Figs. 2, 5). Secondary ureter running parallel to rectum, closely until ureteric interramus where it splits into two branches. Ureteric interramus shallowly excavated. Adrectal branch of secondary ureter continuing straight to mantle collar while abrectal branch turns obliquely exiting in a separate pore. Lung short with alveoli on upper wall parallel to rectum (upper right margin of visceral mass).

Reproductive system

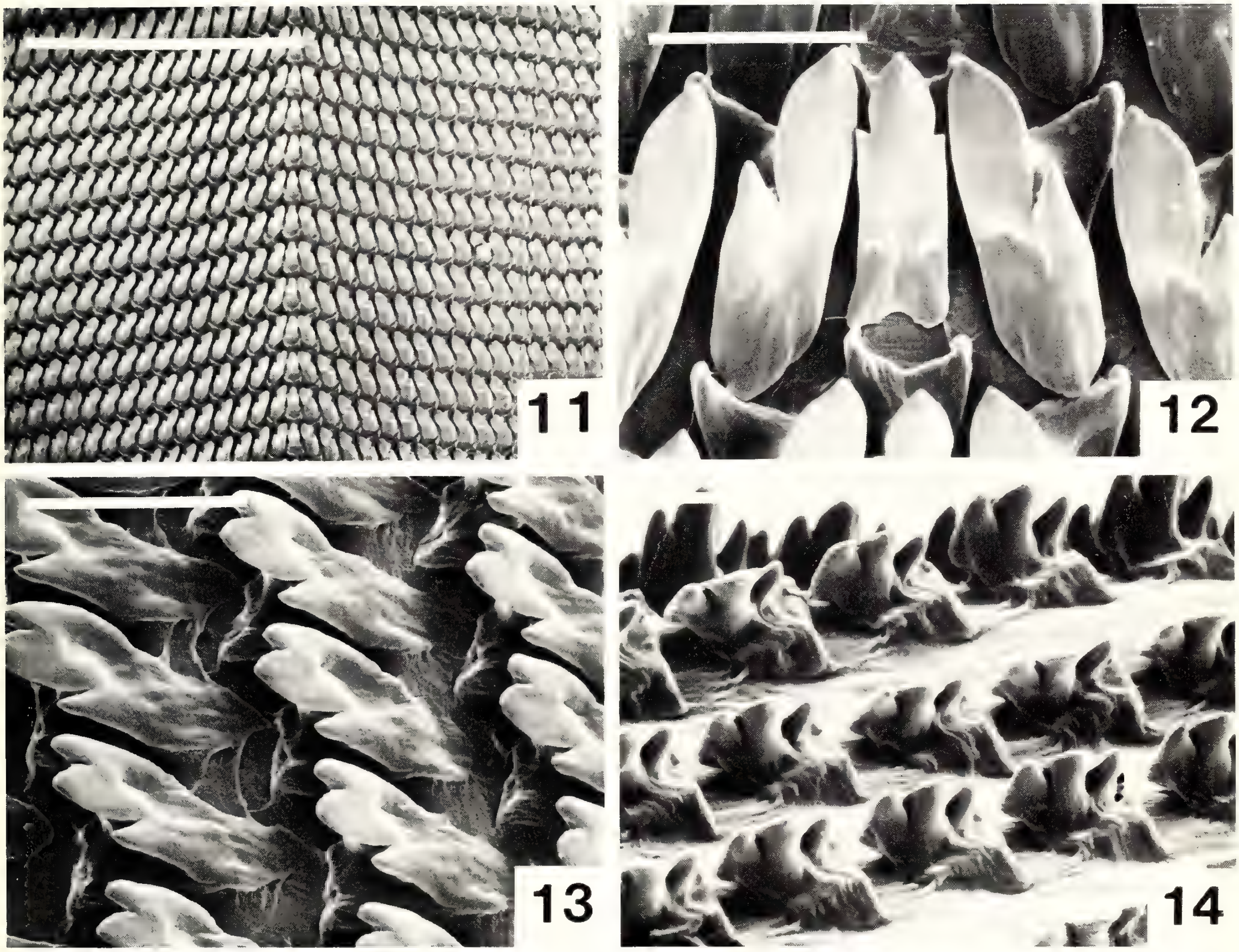
Distal portion of reproductive system (terminal genitalia), in its natural position, running laterally (right margin) to the digestive system. Reproductive pore located on right side of cephalic region, under right ocular tentacle. Medial and proximal portions of system extending dorsally



Figs. 1-5. *Cryptostrakon corcovadensis sp. nov.*, gross morphology and pallial complex. Fig. 1. Dorsal view of the animal. Fig. 2. Ventral view of the visceral mass. Figs. 3-4. Dorsal and ventral views, respectively, of shell of holotype. Fig. 5. Detail of pallial complex. (AG, albumen gland; AL, alveoli; DG, digestive gland; HD, hermaphroditic duct; I, intestine; K, kidney; KL, kidney lobes; M, mantle; MC, mantle collar; MD, mid-dorsal groove; O, ovotestis; PE, pericardium; PN, pneumostome; PU, primary ureter; R, rectum; ST, stomach; UI, ureteric interramus). Scale bars = 2.3 mm (5), 5 mm (2), 8 mm (1, 3-4).



Figs. 6-10. *Cryptostrakon corcovadensis sp. nov.*, reproductive system. Fig. 6. Dorsal view of reproductive system, excluding ovotestis and hermaphroditic duct; penis illustrated in part. Fig. 7. Ventral view of reproductive system as in Fig. 6. Fig. 8. Detail of terminal genitalia. Fig. 9. Penial complex. Fig. 10. Detail of verge. (A, atrium; AG, albumen gland; BC, bursa copulatrix; BCD, bursa copulatrix duct; D, diverticulum; DP, dart papillae; DS, dart sac; F, flagelllum; FO, free oviduct; L, lappets; MG, mucus gland; MGD, mucus gland duct; MS, muscular strands; P, penis; PO, penial orifice; PR, penial retractor; V, vagina; VD, vas deferens; VE, verge). Scale bars = 2.5 mm (6-7), 1 mm (8, 10), 1.5 mm (9).



Figs. 11-14. *Cryptostrakon corcovadensis sp. nov.*, radula. Fig. 11. General view. Fig. 12. Detail of central and lateral teeth. Fig. 13. Dorsolateral view of marginal teeth. Fig. 14. Ventral view of marginal tricupsid teeth. Scale bars = 20 µm (12-14), 200 µm (11).

over esophageal crop; albumen gland located under reduced visceral mass. Almost all of system's wall bright white, contrasting with dark color of esophageal crop content to which it is in part appressed.

Proximal and *Medial Genitalia* (Figs. 2, 6, 7): Ovotestis composed of multiple acini embedded in lobes of proximal digestive gland in visceral mass (Fig. 2). Color of ovotestis pale yellow. Hermaphroditic duct thin and convoluted, descending from ovotestis to Fertilization Pouch-Spermathecal Complex (FPSC) in base of albumen gland, which is small and white. Spermoviduct extending from albumen gland spreading over esophageal crop. Bursa copulatrix duct longer than spermoviduct and running parallel to it. Diverticulum short and thin. Bursa copulatrix sac narrow, and about same diameter as duct.

Terminal Genitalia (Figs. 6, 7, 8): Vagina short, with internal thin longitudinal folds. One dart sac, short and cylindrical, seated on inferior zone of vagina. Internal dart papilla extending from middle to basal part of dart sac (Fig. 8). Mucus gland composed of multiple thin tubules, finger-shaped, opening into one thicker and convoluted duct that ends in middle zone of dart sac. Tubules of mucus gland enveloped by common membranous and transparent tissue giving compact appearance to organ. Tubules of mucus gland folded into two equal portions (Fig. 7). Thin muscular strands connecting dart sac with free oviduct and mucus gland duct. Penial complex composed of thin slender penis continued by short epiphallum (Fig. 9). Verge present, located in upper portion of penis occupying half penial length. External structure of verge consisting of two opposed columns of oblique plaques (Fig. 10). Penial pore (= penial orifice) subterminal, lateral. Penial muscular retractor long and thin, inserting in medial zone of epiphallum. Flagellum as long as penis being coiled over itself. Vas deferens thin, after passing trough angle formed by dart sac and mucus gland duct, inserting on penial complex. Atrium short with internal thin transversal ridges (Fig. 8).

Digestive system

Radula: Central teeth narrow, tricuspid, with tall mesocone, 9-10 bicuspid laterals, and 25 wide, tricuspid marginals (Figs. 11-14).

Jaw: Arched, apparently smooth but fine parallel striae evident (by SEM observations) in medial zone, without ribs; both margins lined by fine denticles.

Buccal mass round. Esophagus short and slender, opening dorsally from anterior buccal mass. Internal ornamentation consisting of longitudinal ridges. Esophageal crop well-developed, appressing terminal portion of reproductive system toward right body wall. Esophageal crop running backward to end of body cavity. Wall of esophageal crop thin, almost transparent to whitish, with irregularly distributed internal pustules. Two salivary glands laterally appressed to esophageal crop. Paired salivary ducts entering buccal mass on dorso-lateral surface on either side of esophagus. Last portion of salivary glands not joining in a common body as observed in other xanthonychids. Stomach and esophageal crop connected by thin tubular portion of digestive tract. Stomach embedded in digestive gland. System ending at rectum in mantle collar.

Central nervous system

Central nervous system forming ring around esophagus. Anterior nerve ring composed of clearly spherical cerebral ganglia. Cerebral commissure short (0.5 mm in length in Holotype). Cerebropedal connectives short (0.75 mm in length in Holotype). Pedal ganglia elongated perpendicular to foot longitudinal axis. Pleuropedal connectives inserting on posterior external surface of pedal ganglia. Visceral chain compact. Visceral ganglion fused with both parietal ganglia. Pleural ganglia in close contact with parietal ganglia.

DISCUSSION

Semislugs are snails in which shell reduction has proceeded so far that the esophageal crop is at least partly contained in the foot cavity and the animal cannot retract inside the shell (Tillier, 1984). Other particular characters found in *Crytostrakon corcovadensis* that, according to Tillier (1983, 1984), could be related with the semislug condition are: (1) stomach retained in the upper visceral cavity; (2) shortening of the lung and development of secondary repiratory structures (alveoli); (3) reproductive system almost entirely contained in the foot cavity (with the exception of ovotestis and hermaphroditic duct); (4) digestive gland occupying most of the reduced visceral cavity and invading the lung cavity; (5) rotation of the axis of the kidney so that this axis tends to be perpendicular to the longitudinal axis of the foot; (6) kidney U-shaped with basal foldings or lobes; and (7) mantle completely concealing the shell. Although some authors (Solem, 1978; Tillier, 1983, 1984, 1989) suggested that these kinds of characters are consequences of the semislug condition, their true significance should be explored only when based upon a phylogenetic hypothesis constructed by strict methodology (Henning, 1966; Farris, 1983).

When Binney established the genus *Cryptostrakon*, he compared it with *Mariella, Gaeotis*, and *Parmella*, stressing that differences were especially strong in the jaw and radula. Besides those comparisons he did not clarify the systematic position of *Cryptostrakon*. Pilsbry (1900: 29) described the genus *Metostracon* and affirmed that "I am somewhat disposed to think that it [*Cryptostrakon*] will be found to agree in the main with *Xanthonyx* or possibly *Metostracon*." However, he stressed that as the shell of *Peltella* and *Gaeotis* (Bulimulidae) were very similar to that of *Cryptostrakon*, the systematic position of *Cryptostrakon* would remain doubtful until at least its genitalia were known. According to Breure (1974) (who described the anatomy of the genus *Gaeotis*), *Peltella, Gaeotis, Pellicula*, and *Amphibulima* constitute a natural group within the Bulimulidae. The anatomical differences with *Cryptostrakon* are so strong that it would be impossible to include it within the Bulimulidae. Concerning families of Helicoidea that also present semislug genera such as Helicarionidae and Vitrinidae, *Cryptostrakon* could not be assigned within these families because: (1) it does not have an esophageal crop (Tillier, 1989), (2) it does have an aulacopod foot and caudal gland, (3) the Vitrinidae are also carnivorous showing an elongated buccal mass, and (4) it presents a substantially different genital system.

Based on the new internal anatomical data provided in this paper, especially the presence of a dart sac and mucus glands homologous (by position) to those in Xanthonychidae, the systematic position of the genus *Cryptostrakon* within the Xanthonychidae is confirmed. Unfortunately because there is no material of *C. gabbi* presently available (other than the type material, that, as stated before, is in poor shape for anatomical studies), it is not possible to point to several anatomical differences between the two species, but only to compare the few characters described by Binney in his original description.

The species *Cryptostrakon corcovadensis*, presently described, was included in the genus because it agrees with Binney's (1879: 258) generic diagnosis, especially in general body shape ("animal slug-like, cylindrical, attenuated behind"), disposition of the mantle ("mantle thin, small, oval, entirely covering the shell"), radula ("central teeth tricuspid, laterals bicuspid, marginals quadrate"), and shell ("shell rudimentary, membranous, without distinction of septa, a spiral arrangement indicated above by depressed lines, below by raised ridges").

Cryptostrakon corcovadensis differs from *C. gabbi*, the other species of the genus, in its body size being smaller, and in the jaw described by Binney (1879: 260) as follows: "Jaw strongly arcuate, ends blunt but little attenuated; anterior surface with two stout decided ribs, denticulating either margin and several other subobsolete ribs." In *C. corcovadensis*, the jaw is almost smooth without ribs. *C. corcovadensis* also differs in shell size. These characters justify the creation of a new species that without doubt belongs to *Cryptostrakon*. No other xanthonychid genera would have received this species. Comparison with other xanthonychid semislug and slug genera revealed that (Table 1): *C. corcovadensis* differs from *Xanthonyx* in: (a) absence of terminal tail horn, (b) shell smaller and more depressed, (c)

Table 1. Selected comparative characters among species of xanthonychid semislug genera.

Characters	*Metostracon mima*	*Xanthonyx* sp.	*Bunnya bernardinae*	*Cryptostrakon corcovadensis*
body shape	slug	semislug	semislug	semislug
tail	without horn	with terminal horn	with terminal horn	without horn
middorsal groove	absent	present	absent	present
shell	one whorl, oblong-oval, 11 mm long	with 2 whorls, spiral	with 2-1/2 whorls, vitriniform, 18 mm in diameter	with 2 whorls, depressed, 8 mm in diameter
body cavity	excavating the tail	not excavating the tail	not excavating the tail	not excavating the tail
jaw	with eight ribs	ribbed	with ten ribs	fine grooves, no ribs
buccal mass	globular	ovoid	ovoid	globular
position of heart	between two lobes of the kidney	to the left of the kidney	to the left of the kidney	partially surrounded by the kidney
ureteric interramus	deeply excavated	shallowly excavated	shallowly excavated	shallowly excavated
lung	with alveoli near the pneumostome	with alveoli near the pneumostome	with alveoli near the pneumostome	with alveoli along the rectum and near the pneumostome
kidney	with 3 lobes	without lobes	without lobes	with 4 short lobes
verge	short, with 3 terminal projections	as long as the penis	as long as penis, with 3 flattened projections	half the penis length with 2 terminal projections
mucus glands	2, inserted in apical portion of dart sac	2, inserted in the vagina where it joins the dart sac	3, inserted in the vagina	one duct with several long finger glands inserted in dart sac
dart sac	one, round, muscular, inserted in vagina	one, inserted in vagina	3, double, round, inserted in vagina	one, cylindrical, seated in the vagina
albumen gland	straight, in pedal cavity	bilobate, in visceral cavity	bilobate, in visceral cavity	curved, in visceral cavity
left parietal ganglion	fused with both visceral and left pleural	in contact, not fused with visceral ganglion and pleural ganglion	fused with visceral ganglion	fused with visceral ganglion and right parietal
position of visceral ganglion respect the medial plane of pedal ganglia	on the left side	median	on the left side	on the left side

jaw not ribbed, (d) buccal mass globular, (e) heart partially surrounded by kidney, (f) kidney with lobes, (g) smaller verge, (h) shape of mucus glands, (i) albumen gland not bilobed, (j) visceral ganglion fused with both parietal ganglia, and (k) general size of the body. *C. corcovadensis* is similar to *Metostracon* in that both genera present lobes in the kidney, an albumen gland not bilobed, a globular buccal mass, and fusion of ganglia in the central nervous system. The differences between *C. corcovadensis* and *Bunnya* appear to be stronger than those with the other two mentioned genera.

Many assumptions have been made about the phylogenetic relationships of *Cryptostrakon*. Pilsbry (1900) stated that *Cryptostrakon* is morphologically closer to *Xanthonyx* than to any other xanthonychid semislug genera. He also suggested that the genera *Xanthonyx*, *Metostracon*, and *Cryptostrakon* should be grouped together in the same subfamily due to their common condition as semislug taxa. This idea was subsequently rejected by Nordsieck (1987) who proposed a new subfamily containing only the genus *Metostracon* which has incompletely reached the slug stage, separate from the Xanthonychinae which contains *Xanthonyx*. Nordsieck also stressed that the differences between *Metostracon* and *Xanthonyx* are so important that parallel evolution of these two groups must be assumed.

Traditionally the lack of an objective, testable analysis on the phylogenetic relationships of the Xanthonychidae genera has caused confusion in their classification. For this reason, to eliminate all arbitrary analysis, only the formulation of cladistic hypotheses based on clearly described characters will produce a predictive classification. As stated by Wheeler (1995: 31), "Such phylogenetic classifications combine the best of descriptive taxonomy and phylogenetic analysis providing the historical perspective essential to a biology that is truly evolutionary."

ACKNOWLEDGMENTS

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Chilina megastoma Hylton Scott, 1958 (Pulmonata: Basommatophora): a study on topotypic specimens

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Abstract: This is a contribution to the knowledge of *Chilina megastoma* Hylton Scott, 1958 (Pulmonata: Basommatophora: Chilinidae), based on the study of topotypic specimens. This species is endemic to the Iguazú Falls, a frontier region between Argentina and Brazil. *C. megastoma* is known only from a brief original description, which was based exclusively on the shell morphology of a single specimen. The relevant results include the following: the radula is formed by 40-50 transverse rows of teeth in "chevron" arrangement; the central tooth is bicuspid with a reduced or rudimentary third cusp; the first lateral is tricuspid with a wide and short base, and the remaining laterals and marginals (40-44) each have four cusps and a long attachment base, which is bent in the marginals. The secondary or accessory seminal receptacle, in the female genital system, is strongly reduced and does not seems to be functional. The penis is as long as the penis sheath, and the penis sheath is two or three times longer than the prepuce.

Key words: *Chilina*, morphology, topotypes, endemic species, Chilinidae

The family Chilinidae comprises only one genus, *Chilina* Gray, 1828, which is distributed exclusively in South America (Castellanos and Gaillard, 1981; Castellanos and Miquel, 1991). About 17 *Chilina* species have been recognized as distributed in Argentina (Castellanos and Gaillard, 1981; Castellanos and Landoni, 1995).

Previous knowledge on the soft-part anatomy of members of the genus mainly comes from the studies of Haeckel (1911) and Duncan (1960a, b; 1975). Recently, Valdovinos and Stuardo (1995), based upon study of the radula, soft-part anatomy, and shell morphology of the 36 species of the genus described from Chile, proposed a new genus, *Archaeochilina*, to include *Chilina angusta* (Philippi, 1860), and recommended the rearrangement of the remaining Chilean species in three new subgenera: *Chilina* s. s., *Mesochilina*, and *Neochilina*. The authors proposed the nervous system organization as providing the only reliable character to afford a basis for supraspecific arrangement.

Argentine species of *Chilina* (16 species with three subspecies) have been studied by Hylton Scott (1958), Castellanos and Gaillard (1981), Castellanos and Miquel (1980), and Miquel (1984; 1986a, b).

Chilina megastoma Hylton Scott, 1958, was described from the Iguazú Falls (25°35' S, 54°35' W), Misiones Province, Argentina. The species is known only from the type locality (Castellanos and Landoni, 1995). The original description was based on the shell morphology of a single specimen, thus, details on soft-part anatomy, especially the nervous system, genital system, and radular features, are completely unknown.

In the present paper, the results of the study on topotypic specimens of *Chilina megastoma*, focusing on its shell morphometry, radular ultrastructure, anatomy of the male and female genital systems, and nervous system, are given. In addition, *C. fluminea* (Maton, 1809), a species widely distributed in Argentina, was used for comparative analysis of several anatomical features.

MATERIALS AND METHODS

Two samples of *Chilina megastoma* were obtained, one from the vertical cliff behind the Salto Dos Hermanas waterfall on the Argentine side of the Iguazú Falls, Misiones, Argentina, and the other from a vertical cliff permanently swept by a small waterfall at the Brazilian side (the Iguazú River is in the northeastern part of Argentina, and it constitutes the natural geographical border with Brazil at that site). Two other samples were collected from Salto San Martín at the Iguazú Falls and from a small waterfall at the Arrechea Rivulet, the latter 2 km away from the type locality.

The specimens were fixed in 10% formalin after having been partly relaxed by immersion in warm water (55°C) for a few minutes. After relaxation and fixation in formalin, the specimens used for anatomical studies were treated and preserved in a modified Raillet-Henry's solution (distilled water 93%, acetic acid 2%, formalin 5%). The radulae were dissected from the buccal mass, then treated with 10% sodium hydroxide (12 hrs), rinsed in distilled water and properly mounted and coated with gold for scanning electronic microscopic observation.

The type specimen of *Chilina megastoma*, deposited at the Museo de la Plata (MLP, without registration number), has also been studied. Specimens of *C. fluminea* used for comparative purposes were collected from the sandy shore at the Río de La Plata River and from Miguelín Rivulet, both at Punta Lara, Ensenada, Buenos Aires.

Shell measurements (total shell length, last whorl length, major and minor diameter of the shell aperture) were taken under a stereoscopic microscope provided with a micrometer eyepiece.

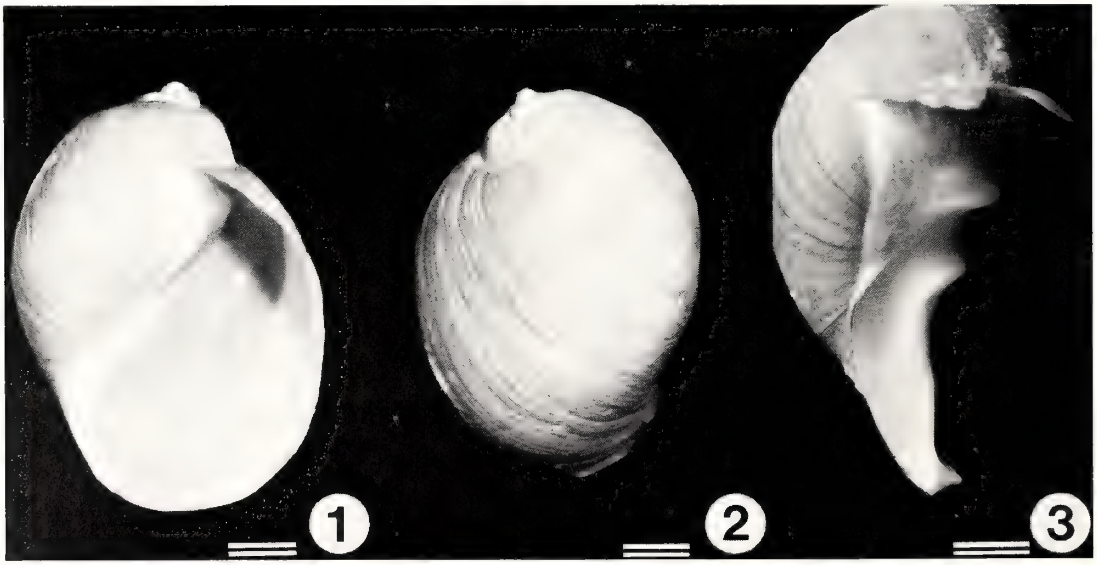
Voucher specimens were deposited at the malacological collection at Museo de La Plata (MLP nos. 5098, 5099, 5128, 5129, 5130, 5246, and 5261).

RESULTS

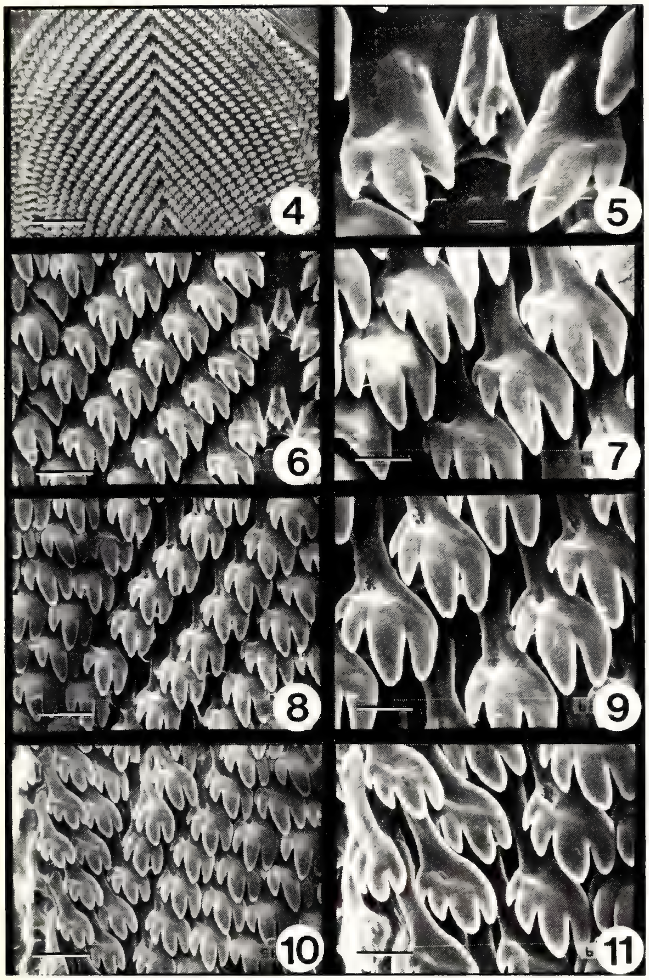
SHELL: The diagnostic features of *Chilina megastoma* are shape and development of the spire (extremely low) and the great development of the last whorl of the shell, resulting in a large aperture of the shell (Fig. 1). The ratio of last whorl to shell length ranged from 0.92-0.97 (mean 0.95, standard deviation 0.01). The aperture is oval; minor diameter to major diameter ratio ranged from 0.53-0.87 (mean 0.67, standard deviation 0.08). The shell is yellowish, olivaceous, or in some cases dark brown. The surface is smooth, only sulcated with weak radial periostracal striae and helicoidal stripes, particularly evident near the suture, which is deeply marked (Fig.2). The columellar border is straight or slightly curved, somewhat concave, with a marked fold at its upper end (usually referred to as the columellar tooth). The parietal area has a thin white callus. A weak fold of the parietal area forms an indistinct lamella, also referred to in the literature as a "parietal tooth" (Hylton Scott, 1958; Castellanos and Gaillard, 1981), lying slightly over the columellar fold (Fig. 3). The outer lip is sharp and evenly curved.

DIGESTIVE SYSTEM: The general morphology of the digestive system follows the pattern already known in the genus (Haeckel, 1911; Harry, 1964). The posterior end of the radular sac exceeds the posterior end of the buccal bulb, forming a short tube slightly bent dorsally. The salivary glands are not fused at their posterior end.

The radula is composed of approximately 40 sharply oblique rows of teeth (Fig. 4), that is, the tooth rows are arranged "in chevron." The angle between the right and left halves of each row is about 90°. The central tooth is asymmetrical, bicuspid, with an additional third cusp, greatly reduced, and partly overlapping the major cusp (Fig. 5). The attachment base of the central tooth is elongated, deltoid in shape, with the proximal end concave.



Figs. 1-3. Shell of *Chilina megastoma* Hylton Scott, 1958. Fig. 1. Apertural view (MLP 5098). Fig. 2. Dorsal view, showing the periostracal sculpture (MLP 5098). Fig. 3. Detail of the columellar area, columellar fold, and parietal lamella (MLP 5099). Scale bars = 2 mm.



Figs. 4-11. Radula of *Chilina megastoma* Hylton Scott, 1958. Fig. 4. General view showing the typical arrangement of teeth. Fig. 5. Central tooth and first lateral teeth. Figs. 6, 8, 10. Lateral teeth (from central toward marginals). Figs. 7, 9, 11. Details for Figs. 6, 8, 10, respectively. Scale bars = 10 µm (Fig. 5), 25 µm (Figs. 7, 9, 11), 50 µm (Figs. 6, 8, 10), 250 µm (Fig. 4).

The major cusp is relatively short, cylindrical, and pointed at the tip. The minor cusps are very short and thorn-like. The first lateral tooth is tricuspid, with a short, wide, and strong base. The mesocone has a short dagger-like shape, somewhat directed toward the central tooth. The entocone and ectocone are short, triangular, and nearly symmetrical. The entocone is somewhat larger than the ectocone (Figs. 5-6). The second lateral tooth is also tricuspid, but with a slender, longer, and slightly asymmetric attachment base. The mesocone is the largest cusp, and the ectocone markedly smaller than the entocone (Fig. 6). From the third lateral tooth to the marginal teeth, the morphology of the teeth is relatively similar. The free plate of the teeth is palmate with four cusps; the attachment base is slender, and it joins the free plate at a point slightly shifted toward the minor cusps. In the last marginal teeth, the attachment base becomes slender, obliquely inserted, and slightly bent (Figs. 7-11).

REPRODUCTIVE SYSTEM: Regarding the general aspects, the features of the female and male genital systems are coincident with those previously described for the genus (Haeckel, 1911; Duncan, 1960a, b, 1975). Thus, only the diagnostic characteristics for *Chilina megastoma* will be described.

Female genital system: The seminal receptacle or spermatheca is oval, located at the left side of the visceral mass, lying just below the ventricle. A long duct runs transversely across the visceral mass, passing over the utero-vaginal complex, to connect the seminal receptacle with the distal end of the vagina just before the female genital opening at the right side of the body (Figs. 12-13). Another duct arises posteriorly, just between the vagina and the uterus,

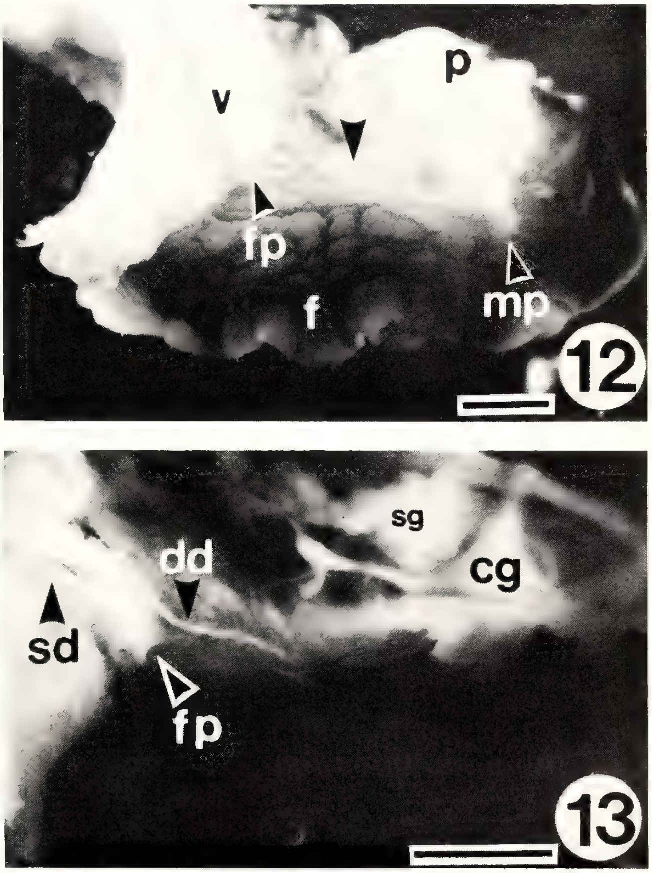
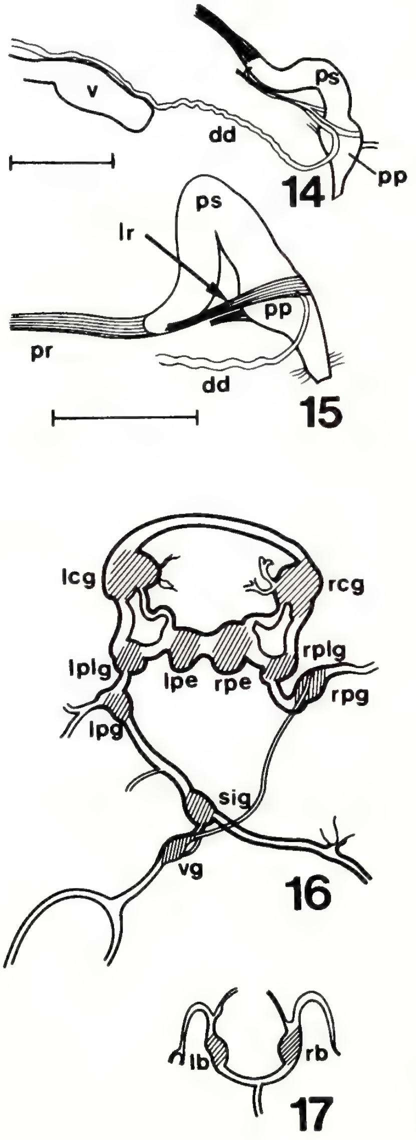


Fig. 12-13. *Chilina megastoma* Hylton Scott, 1958. Fig. 12. Lateral view of a partially dissected specimen showing the terminal portion of male and female genital systems, and the deferent duct (arrow heads) dissected from the body wall (MLP 5210). Fig. 13. Details of the terminal female genital system showing the spermathecal duct crossing over the vagina to open at its distal end (MLP 5210). (cg, cerebral ganglion; dd, deferent duct; f, foot; fp, female pore; mp, male pore; p, penis; sd, spermathecal duct; sg, salivary gland; v, vagina).

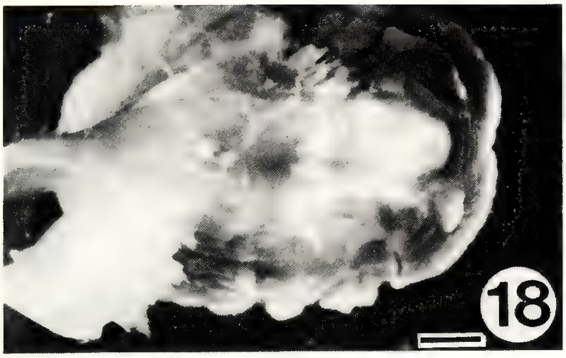
called a secondary or accessory seminal receptacle. This duct runs closely adhered to the wall of the uterus, and it is barely enlarged at the tip to form a nearly indistinguishable, rounded ampulla.

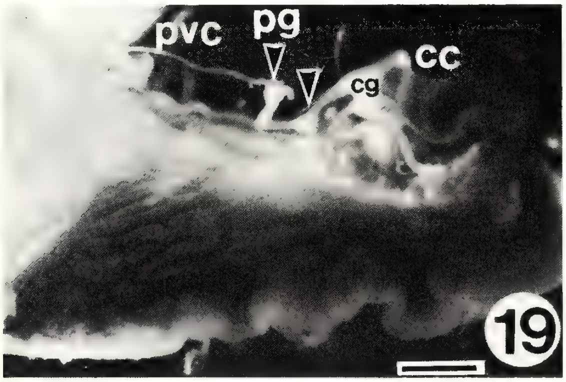
Male genital system: The deferent duct arises as a separate male genital duct only after traversing the prostate, running below the uterus and the vagina. Near the distal end of the vagina the deferent duct emerges from the haemocoelic space, sometimes forming a very small loop, entering into the muscular body wall (Figs. 12-13). The distal portion of the deferent duct is very sinuous running along the right side of the body wall (Figs. 12-14), close to the surface and immediately below the external reproductive groove which, arising just at side of the female genital pore, runs toward the base of the right tentacle close to the male genital system opening. At this point, the deferent duct diverges from the body wall coming into the cephalopedal haemocoel and turning posteriorly toward the posterior end of the penis sheath, the site at which it enters the penis (Fig. 15). The penis sheath is a slender and nearly cylindrical tube (Fig. 14). The penis is slender, as long as the penis sheath; its surface is rugose due to the presence of deep transverse furrows and padded ridges or short lamellae. At the anterior half of the penis, these ridges are more or less longitudinally arranged. The prepuce is a somewhat triangular or cordiform structure with thick muscular walls (Figs. 14-15). The prepuce is shorter than the penis sheath; its length is half or a third of that of the penis sheath. A powerful penis sheath retractor muscle arises from the posterior end of the penis sheath (Fig. 15), and it is attached at the columellar muscle. Another series of four or five muscular bundles are attached to the lateral wall of the prepuce. Two long muscular bundles are joined to the frontal wall of the prepuce running on each side, and connecting with the penis sheath retractor. The contraction of these two muscles determines the erection of the penial complex (Figs. 14-15). The deferent duct runs over the right-most muscular bundle, toward the proximal end of the penis (Figs. 14-15).

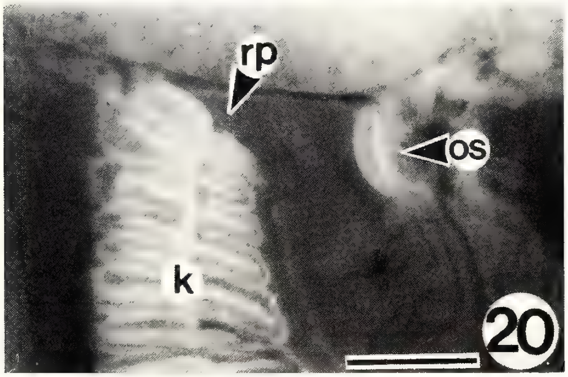


Figs. 14-17. *Chilina megastoma* Hylton Scott, 1958. Genital and nervous systems. Fig. 14. Camera lucida drawing of the distal portion of the female and male genital systems. Fig. 15. Camera lucida drawing with details of the penial complex. Fig. 16. Semi-diagramatic scheme of the anterior and posterior nerve rings. Fig. 17. Semi-diagramatic scheme of the innervation of the buccal mass. Scale bars = 1 mm. (dd, deferent duct; lb, left buccal ganglion; lcg, left cerebral ganglion; lpe, left pedal ganglion; lpg, left parietal ganglion; lplg, left pleural ganglion; lr, lateral penis retractor; pp, prepuce; pr, penis retractor; ps, penis sheath; rb, right buccal ganglion; rcg, right cerebal ganglion; rpe, right pedal ganglion; rpg, right parietal ganglion; rplg, right pleural ganglion; sig, subintestinal ganglion; v, vagina; vg, visceral ganglion).

NERVOUS SYSTEM: The cerebral and pedal ganglia are joined by commissures and connectives to form the anterior nerve ring located at the anterior half of the buccal mass, just at or a little behind the origin of the esophagus (Figs. 16, 18-19). However, the location of the anterior nerve ring showed considerable variation according to the degree of retraction of the buccal mass, but it was always located at the anterior half of the buccal mass. The right pleural ganglion is joined by somewhat short connectives to both cerebral and pedal ganglia. A relatively long connective joins the right pleural ganglion to the right parietal ganglion (supraesophageal ganglion of Fretter, 1975), which gives off a large nerve that supplies the osphradium (Fig. 19), and a second very long and thin connective that runs to the visceral ganglion (also called abdominal [Fretter, 1975] or innominate [Harry, 1964]), at the end of the posterior nerve ring. On the left side of the posterior nerve ring, two short connectives join the left cerebral and pedal ganglia with the pleural ganglion, from which a relatively short

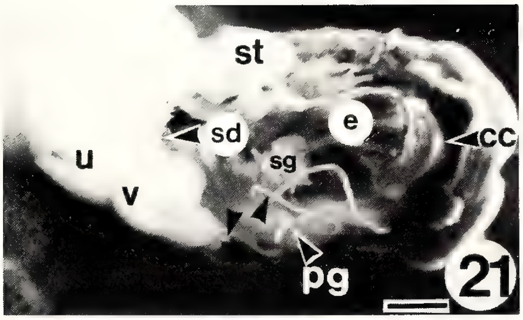


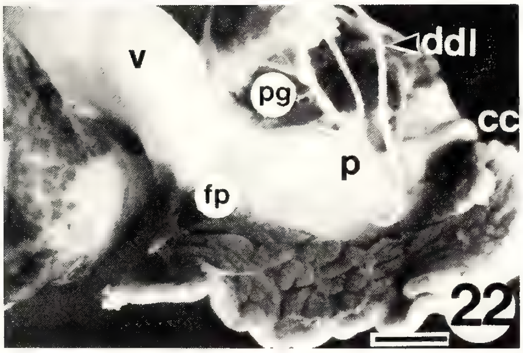


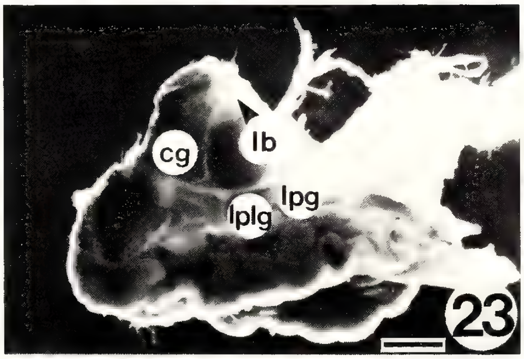


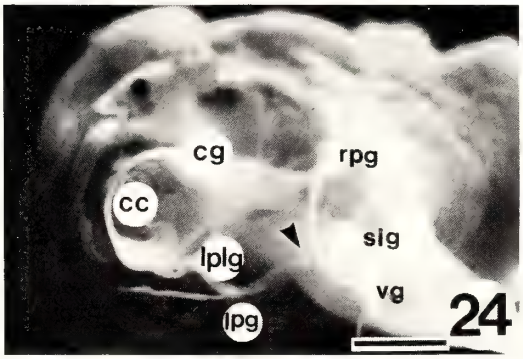
Figs. 18-20. *Chilina megastoma* Hylton Scott, 1958 (MLP 5210). Fig. 18. Dorsal view of a dissected specimen to show the anterior and posterior nerve rings (see Fig. 16 for reference). Fig. 19. Lateral view showing the location of the cerebral commissure. Posteriorly, the cerebral ganglion, the right pleural ganglion (arrowhead), the right parietal ganglion and the parietovisceral connective, are shown. Fig. 20. View of the mantle roof showing the location of the osphradium close to the renal pore at the right end of kidney. Scale bars = 1 mm. (cc, cerebral commissure; cg, cerebral ganglion; k, kidney; os, osphradium; pg, parietal ganglion; pvc, parietovisceral connective; rp, renal pore).

connective arises and runs toward the left parietal ganglion. A long connective joins the left parietal ganglion to the subintestinal ganglion, which lies over the columellar muscle, at its posterior half. A somewhat large nerve arises at a point approximately 2/3 the length of the left parietal-subintestinal connective. There is a slight swelling just at the site where that nerve originates; however, it might not









Figs. 21-24. *Chilina fluminea* (Maton, 1809) (MLP 5246). Fig. 21. Dorsal view of a partially dissected specimen showing position of the cerebral commissure, and the long loops of the deferent duct (arrowheads). Fig. 22. Right lateral view. Fig. 23. Left lateral view. Fig. 24. Dorsal view of the nervous system. Scale bars = 1 mm. (cc, cerebral commissure; cg, cerebral ganglion; ddl, deferent duct loop; e, esophagus; fp, female pore; lb, left buccal ganglion; lpg, left parietal ganglion; lplg, left pleural ganglion; p, penis; pg, parietal ganglion; rpg, right parietal ganglion; sd, spermathecal duct; sg, salivary gland; sig, subintestinal ganglion; st, spermatheca; u, uterus; v, vagina; vg, visceral ganglion).

definitely be considered a ganglion. Finally, a very short connective joins the subintestinal ganglion to the visceral one, closing the posterior nerve ring. From the latter, a large nerve arises, and shortly thereafter, it gives off two nerves which supply the visceral mass. From the subintestinal ganglion, a large nerve runs toward the right, passing through the columellar muscle to innervate the distal part of the vagina and the pneumostomal appendage. As is characteristic for the genus, the pleuro-visceral connectives show incomplete torsion (Figs. 16, 18).

At each side of the buccal mass (dorsolaterally), far removed, and behind the origin of the esophagus, are the buccal ganglia. The ganglia are joined by a somewhat short commisure which passes below the esophagus. A nerve, arising from the middle of this commisure, sinks into the buccal mass shortly after its origin (Fig. 17). The buccal ganglia are connected to each cerebral ganglion by long connectives, forming an U-shaped open nerve ring. The osphradium, located on the roof of the mantle cavity, close to the renal pore, and at the anterior end of the pneumostome, is a flat oval-shaped organ formed by an slightly curved furrow, bordered by two elongated and inflated lips (Fig. 20).

DISCUSSION

From the present knowledge of the distribution of *Chilina megastoma*, the species seems to be endemic to a reduced number of environments closely related to high energy freshwater courses such as vertical cliffs and rocky walls permanently swept by winds and water trickles from nearby waterfalls. As reported for other gastropod species (Trussell *et al.*, 1993), the extreme reduction of the spire, the globose shape of the last whorl and the wide aperture of the shell shown by *C. megastoma* represent adaptive responses to particular environmental conditions, such as dislodgement agents in high energy watercourses.

Hylton Scott (1958), and Castellanos and Gaillard (1981) considered that *Chilina megastoma* approaches *C. fluminea* in shell morphology, pointing to the lack of periostracal sculpture as a differential character in the latter.

With respect to the anatomy of soft parts, several differences should be pointed out. The general features of the genital system of *Chilina megastoma* are coincident with those described by Miquel (1984) for *C. fluminea* and by Harry (1964) for *C. fluctuosa* Gray, 1828. The following differences have been observed: in *C. megastoma* the duct of the seminal receptacle or spermatheca crosses, from left to right, above the lower portion of the female genital complex, just below the floor of the pallial cavity, while in *C. fluminea*, the duct passes over the anterior border of the distal part of the spermoviduct and prostate, and then turns downward, just at the point where the uterus connects with the vagina (Fig. 21). Thereafter, to traverse the vagina ventrally, the duct reappears dorsally and immediately opens near the tip of the vagina. The same features (Fig. 16) have been illustrated by Harry (1964) for *C. fluctuosa* from Chile. Moreover, in *C. megastoma*, the accessory or secondary seminal receptacle is barely enlarged at the tip, while in *C. fluminea* the tip of this organ is greatly enlarged into a large pear-shaped bulb. This difference in shape and size does not seem to be related to organ physiology.

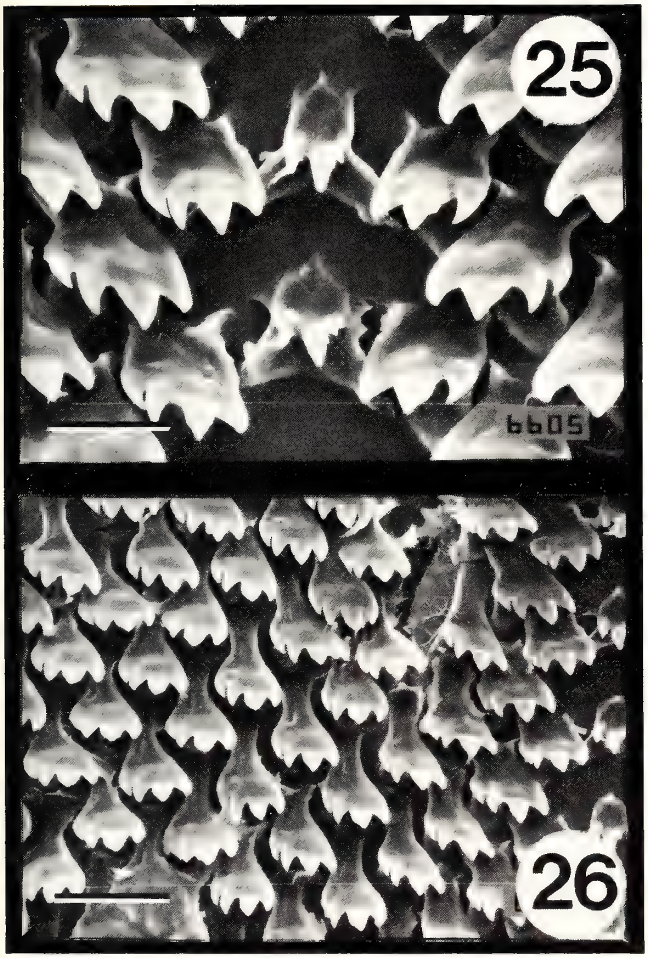
In the male portion of the genital system, several differences among species should be pointed out: while in *Chilina megastoma* the segment of the deferent duct which runs into the muscular body wall just below the genital groove is markedly sinuous (Figs. 12, 14), in *C. fluminea* it is nearly always straight or faintly sinuous (Fig. 22). In addition, in *C. fluminea*, when the deferent duct reenters into the haemocoelic space toward the penis, it develops two or three large loops prior to running along the penial retractor muscle and entering the penis (Figs. 21-22). Unlike this, in *C. megastoma* the deferent duct is strictly as long as the genital organs it accompanies (Figs. 12, 14-15).

According to Valdovinos and Stuardo (1995), the nervous system is the only reliable feature to differentiate Chilean species and to build suitable supraspecific arrangements. According to Haeckel (1911), the structural pattern of the posterior nerve ring seems to be a specific characteristic, especially with respect to the number of ganglia and the length of the connectives between the left pleural and subintestinal ganglia.

The general pattern of the nervous system of *Chilina megastoma*, here studied, does not differ from that described by Harry (1964) for *C. fluctuosa*. However, Harry (1964) was unable to find the left parietal ganglion already described by Haeckel (1911). In the present study, the left parietal ganglion was easily identified in both *C. megastoma* and *C. fluminea* (Figs. 18, 23-24). In *C. megastoma*, the lengths of the left cerebropleural connective and left pleuroparietal connectives were similar. The left parietal-subintestinal connective was approximately three times as long the pleuroparietal connective (Fig. 18). In *C. fluminea* the left pleural and parietal ganglia are closer than they are in *C. megastoma*, being, in some cases, nearly contiguous (Fig. 24). In *C. megastoma* the anterior nerve ring tends to be located more posteriorly than in *C. fluminea*, in which it has always been observed at the anterior end of the buccal bulb (Figs. 21-23).

As in *Chilina fluminea*, the posterior end of the radular sac of *C. megastoma* is projected from the buccal mass, forming a short cylindrical tube bent slightly dorsally. This fact is in contrast with the description given by Harry (1964) for *C. fluctuosa* from Chile.

Several details in morphology of the radular teeth



Figs. 25-26. Radula of *Chilina fluminea* (Maton, 1809). Fig. 25. Details of central and first lateral teeth. Fig. 26. Lateral teeth. Scale bars = 25 µm (Fig. 25), 50 µm (Fig. 26).

seem to differ consistently among species. In *C. megastoma*, the central tooth has a definite triangular, elongated base, while in *C. fluminea* the attachment base is shorter and stronger. The lateral and marginal teeth of *C. megastoma*, have more slender attachment bases and their cusps are longer, with a more definite dagger-like shape than those of in *C. fluminea* (Figs. 25-26). In addition, the last marginal tooth of each row in *C. megastoma* has four cusps (Figs. 10-11), while in *C. fluminea* it usually has six or seven cusps (Castellanos and Gaillard, 1981). The number of tooth rows also differs: 40 in *C. megastoma* and between 60 and 65 in *C. fluminea* (Castellanos and Gaillard, 1981).

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The role of prey mobility in the population ecology of the nudibranch *Cuthona nana* (Gastropoda: Opisthobranchia)

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Abstract: The aeolid nudibranch *Cuthona nana* (Alder and Hancock, 1842) was studied in relation to its specific prey, the hydroid, *Hydractinia polyclina* (Agassiz, 1862). In this predator-prey association, the predator's spatial patterns and behavior, along with prey mobility, could play an important role in maintaining this nudibranch population. The seasonal abundances of *C. nana* documented in this study agree with the sub-annual life cycle previously presented. Both adult and juvenile nudibranchs were present on colonies during most months sampled in 1986-1988. The low prevalence of nudibranchs on hydroid colonies, the behavior of adult *C. nana* (leaving colonies periodically), regeneration in the hydroid, and prey mobility appear to be crucial in maintaining this unique species-specific, predator-prey association. Most nudibranch prey are non-mobile while *H. polyclina* grows on gastropod shells occupied by hermit crabs of the genus *Pagurus*. Adult *C. nana* repeatedly leave the hydroid colonies both in the field and laboratory to lay egg masses, while juveniles spend extended periods of time on the colony and leave only when they approach sexual maturity. The adult behavior of leaving colonies to lay egg masses does not severely jeopardize the newly hatched nudibranchs' probability of finding food. Hermit crab mobility is high and within a 24-hr period crabs frequently pass a given area containing juvenile nudibranchs. Juveniles encounter the hydroid's streaming gastrozooids sweeping over the substratum. *C. nana* undergoes non-pelagic lecithotrophic development from yolk-rich eggs with individuals hatching as crawling juveniles. The predator-prey dynamics between *C. nana* and mobile colonies of *H. polyclina* are similar to those seen in host-parasite associations.

Key words: Hydroidea, Nudibranchia, population ecology, predator-prey, Aeolidoidea, Hydractinia

Few studies describe nudibranch predator population parameters in relationship to their prey (Potts, 1970; Harris, 1973; Todd, 1979, 1981, 1983). Most nudibranch population studies show seasonal population fluctuations and suggest that physical (temperature, wave action) or biological factors (prey availability, competition, predation) control such fluctuations (reviews: Harris, 1973; Todd, 1981, 1983). Aeolid nudibranchs (Gastropoda, Opisthobranchia) often are abundant in seasonal hydroid communities; the communities are sessile and seasonal due to heavy grazing by predators, or because of changes in temperature (Miller, 1961; Thompson, 1964; Fager, 1971; Clark, 1975; MacLeod and Valiela, 1975; Nybakken, 1978; Harris and Irons, 1982).

Unlike most hydroids, the colonial gymnoblastic hydroid *Hydractinia polyclina* (Agassiz, 1862) (formally known as *H. echinata* Fleming, 1828; Buss and Yund, 1989) is a persistent food source throughout the year and is a mobile prey. In the southern Gulf of Maine, *H. polyclina* grows primarily on shells occupied by the hermit crab *Pagurus acadianus* (Benedict, 1901) and less frequently on the shells occupied by *P. arcuatus* (Squires, 1964). No studies have considered how hermit crab movement affects the accessibility of predators to epifaunal prey organisms on their shells. Knowing the probability of a hermit crab passing a given area on the benthos would provide information on prey availability for a predator and how that could impact the population ecology.

The aeolid nudibranch *Cuthona nana* (Alder and Hancock, 1842) (Aeolidoidea) is a hermaphroditic opisthobranch that feeds specifically and exclusively on *Hydractinia polyclina*. Sexually mature *C. nana* leave crab shells bearing hydroid colonies to mate and lay egg masses (Rivest, 1978; Folino, 1993). Relocating a colony for further feeding is a unique challenge for *C. nana* compared to other hydroid feeders. Non-pelagic lecithotrophic larvae hatch and are picked up by the gastrozooids (feeding polyps) of the passing hydroid colony (Rivest, 1978). The movement of the hermit crab is therefore important in bringing the prey, *H. polyclina*, to the slow-moving predator, *C. nana*.

This study documents the localized dynamics of *Cuthona nana* and its prey off of the coast of Maine and expands on shorter previous studies by Rivest (1978) and Folino (1985). The purpose of this study was three-fold: (1)

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to describe the population structure of *C. nana* by documenting nudibranch densities, distributions, and size frequencies on shells with colonies from May 1986 to May 1988 at Gosport Harbor, Maine; (2) to compare juvenile and adult movements on and off *Hydractinia polyclina* colonies; *i. e.* how this behavior affects the location of mobile prey on hermit crab shells; and (3) to estimate prey accessibility for *C. nana* by determining the degree of movement by hermit crabs with hydroids on their shells within a given area. Does crab mobility have an impact on prey availability which could regulate *C. nana* populations?

METHODOLOGY

Collection of animals

Specimens of *Cuthona nana* and colonies of *Hydractinia polyclina* were collected using SCUBA from Gosport Harbor (Haley Cove, Isles of Shoals, Maine), ca. 9.5 km off the New Hampshire coast (42°59' N; 70°36' W). Hermit crabs occurred mainly in the sandy portions of the harbor at depths of 5-10 m.

Population data for *Cuthona nana* was obtained from 23 monthly collections (May 1986 to May 1988) of 21 to 90 hermit crabs, each with colonies covering ca. 100% of the avaiable shell surface. The coverage is approximate because the shell scrapes the bottom when the crab crawls, preventing colony growth. Hydroid-covered shells were placed in individual containers in the field to insure that nudibranchs remained on their original colonies. The containers were either plastic jars (125 ml) or mesh containers (Toby Tea-boys, mesh size ca. 164 µm; Daniel Peikin Company, Silver Spring, Maryland). Although efforts were made to collect shells of various sizes with *Hydractinia polyclina*, larger shells were more visible, creating a sampling bias towards larger shells. Colonies were later examined for nudibranchs using a dissecting microscope.

Nudibranch population structure

For each hydroid-covered shell (therefore for each hydroid colony) the density of nudibranchs and their sizes were recorded. The number of *Cuthona nana* was used to determine the abundance of nudibranchs per colony. Indices of dispersion were calculated to determine the degree of aggregation of nudibranchs on colonies. The dispersion pattern was further analyzed by making comparisons to a negative binomial distribution (Ludwig and Reynolds, 1988; Krebs, 1989). Co-occurrence of nudibranchs was determined by scoring the size of each nudibranch collected on a *Hydractinia*-covered shell versus the number of conspecifics present on the same colony. Individuals were scored as being alone, paired, or with three or more nudibranchs. The nudibranchs were divided into non-reproductive (< 9 mm) or reproductive (> 9 mm) groups based on anatomical and behavioral aspects of reproduction (Rivest, 1978; Folino, 1993).

To determine if colony size affected the number of nudibranchs present on a colony, correlation coefficients were calculated for nudibranch number and colony size for each month sampled. The surface area of a given shell was determined using its linear dimensions following a technique modified (see Folino, 1993) from that used by Shenk and Karlson (1986). Colony size was then estimated from shell surface area. In months when there were small numbers of colonies with nudibranchs present, the data were pooled.

Histograms of nudibranch length frequencies were used to examine the age-class structure by month and were compared for yearly differences using Kolmogorov-Smirnov tests (Sokal and Rohlf, 1981). Monthly size-class histograms (See Folino, 1989) were summarized by grouping individuals into non-reproductive individuals (< 9 mm) and reproductive individuals (> 9 mm) as previously mentioned.

Measuring nudibranch movement

A laboratory experiment was designed to determine differences between juvenile and adult *Cuthona nana* in movement on and off hydroid-colonized hermit crab shells. Hermit crabs were maintained in trays (76 x 64 x 9 cm) filled with sand from Gosport Harbor. Beetags (from Chr. Graze KG, West Germany) were used to label shells so that individual colonies could be assessed for the presence or absence of nudibranchs. Because nudibranchs are difficult to tag, tagging the shells was a way of monitoring nudibranch movement (*i. e.* whether a nudibranch was present or absent since the previous observation). Each tray contained ten tagged hermit crabs and four nudibranchs. Tray densities were chosen from the highest field densities recorded from cofferdam samples taken in May 1987. [A cofferdam is a metal cylinder (0.153 m^2) placed on the benthos to prevent hermit crabs in the enclosed area from escaping before being counted.]

Two trials with two replicate trays were conducted using adult nudibranchs of 12-20 mm. Each adult trial lasted 12 days, and the shells were examined twice daily for the presence or absence of nudibranchs, once in early morning and once in late afternoon to account for night and day activity. Nudibranch movement was measured by calculating the mean number of moves (or change in nudibranch number) on or off a colony per day. A similar experiment was conducted using juvenile (2-4 mm) nudibranchs. One trial of juveniles consisting of four replicated trays was conducted for 21 days.

Measuring hermit crab mobility

Hermit crab mobility was estimated by deploying pitfall traps (Uetz and Unzicker, 1976) on the bottom of Gosport Harbor to sample crabs passing a given area in a 24-hr period. The traps were plastic containers ca. 11 cm in diameter and 15 cm deep. Two grids (each measuring 5 x 5 m) were used to randomly position 20 pitfall traps; holes for the containers were dug in the sand using an airlift. Each container was marked with a numbered flag to insure relocation. Trials were performed monthly from January through May 1988; April was excluded due to rough seas. A given trial consisted of leaving the traps uncovered for 24 h from mid-morning to mid-morning. Traps were emptied and crabs from a given trap were placed in a mesh bag. The number of crabs caught per trap and the presence or absence of *Hydractinia polyclina* colonies on the shells of each crab were recorded. Traps were covered between trials; a few traps became filled with sand and could not be relocated during two of the four trials.

RESULTS

Nudibranch densities

Monthly collections of crabs with hydroid-covered shells provided seasonal estimates for the population of *Cuthona nana* at Gosport Harbor. The mean number of nudibranchs per colony collected from May 1986 to May 1988 demonstrated seasonality of densities, with maxima in April, May, and August, and minima in October and November (Fig. 1). The greatest mean number per shell occurred in May 1987 (2.380; SE = 0.386) and most of those individuals (87%) were < 4 mm in length.

The percentage of hydroid-covered shells with one or more *Cuthona nana* fluctuated over the 23 mo sampled. The greatest percentages of occupied shells for 1986 were in July and August with 55% and 56%, respectively, while April and May showed the greatest percentage of colonies with nudibranchs in 1987 and 1988 (Fig. 2). The percentage of shells with nudibranchs declined in late summer and early fall in 1986 and 1987, and began to increase in November and December in both years. The percentage of shells with nudibranchs increased in the colder months suggesting an increase in population numbers (Fig. 2).

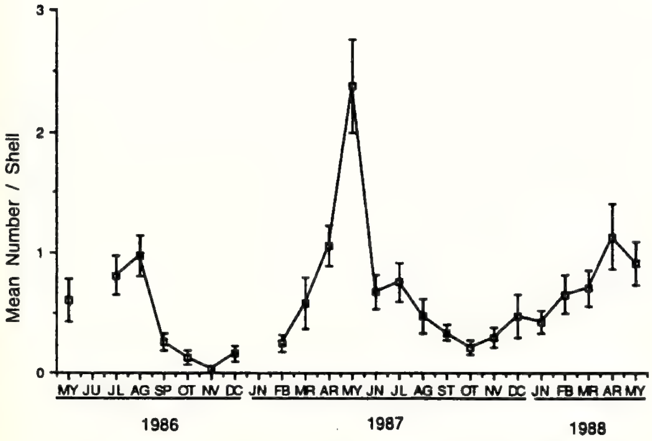

Fig. 1. The mean number of *Cuthona nana* per hydroid-covered hermit crab shell for each month sampled from May 1986 to May 1988. The bar for each mean represents standard error.

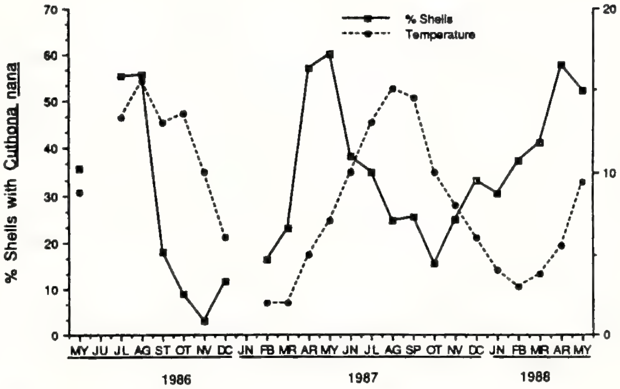

Fig. 2. The percentage of hermit crab shells covered with *Hydractinia polyclina* having one or more *Cuthona nana* present, plotted with temperature.

Nudibranch distributions

Nudibranchs demonstrated an aggregated rather than random dispersion pattern on colonies for all months sampled; tests could not be performed for October, November, and December 1986, and November 1987 because of small sample sizes (Table 1). All indices of dispersion (variance/mean number of nudibranchs per colony ratios) were greater than 1.0, providing evidence for aggregated distributions (Krebs, 1989) (Table 1). A large number of colonies had no nudibranchs, and the ranges of distribution varied by month. In May 1987 and April 1988 a greater range of frequencies was observed; some colonies had as many as 11-19 nudibranchs per colony. Eight out of nineteen months fit the negative binomial distribution indicating strong patterns of aggregation during those months (Table 1).

Numbers of nudibranchs and colony size were not significantly correlated for any of the years sampled (1986: r = -0.080, N = 84; 1987: r = 0.068, N = 241; 1988: r = 0.015, N = 128, p > 0.50 in all cases). Correlation coefficients were also determined for shell size (*i. e.* colony size for completely covered shells) and the number of non-reproductive nudibranchs to test the assumption that larger colonies acquired more juvenile nudibranchs (from the benthos). Again, no significant correlations were obtained

Table 1. The indices of dispersion (variance:mean ratios) and the negative binomial distribution statistics for the number of hydroid-covered shells with 0-19 *Cuthona nana* from May 1986 to May 1988. (k, exponent of the negative binomial distribution; N, number of sample units or hermit crabs; P, probability for the calculated X^2 values of the negative binomial distribution; $S^2 / \overline{X}$, variance to mean ratio; $\overline{X} \pm$ SE, mean plus or minus standard error; X^2, goodness-of-fit of the negative binomial distribution; *, significant fit to the negative binomial distribution).

	MONTH	$S^2 / \overline{X}$	N	$\overline{X} \pm$ SE	k	X^2	P (df = n - 3)
1986	MAY	1.50	28	0.607 + 0.181	0.73	1.45	P < 0.100 (1)
	JUL	1.25	38	0.816 ± 0.164	16.0	9.17	P < 0.010 (2)*
	AUG	1.23	45	0.978 ± 0.164	2.0	0.91	P < 0.250 (2)
	SEP	1.48	84	0.262 ± 0.068	0.37	1.54	P < 0.100 (1)
	OCT	1.56	68	0.132 ± 0.055	---		
	NOV	1.00	33	0.030 ± 0.030	---		
	DEC	1.46	61	0.164 + 0.063	---		
1987	FEB	1.55	73	0.247 ± 0.072	0.30	1.28	P < 0.250 (1)
	MAR	3.97	52	0.519 ± 0.199	0.21	13.17	P < 0.025 (6)*
	APR	1.76	63	1.060 ± 0.172	2.00	19.49	P < 0.001 (5)*
	MAY	5.64	90	2.380 ± 0.386	0.54	19.79	P < 0.100 (16)
	JUN	1.83	63	0.683 v 0.141	0.79	1.97	P < 0.250 (2)
	JUL	3.03	87	0.759 ± 0.163	0.44	8.44	P < 0.100 (6)
	AUG	2.96	65	0.477 ± 0.147	0.28	14.70	P < 0.010 (6)*
	SEP	1.34	98	0.337 ± 0.068	1.10	2.94	P < 0.100 (2)
	OCT	1.47	83	0.217 ± 0.062	0.62	0.25	P < 0.500 (1)
	NOV	1.04	44	0.295 ± 0.083	---	---	
	DEC	1.40	21	0.476 + 0.178	1.30	0.88	P < 0.250 (1)
1988	JAN	1.26	56	0.429 ± 0.098	1.10	0.695	P < 0.250 (1)
	FEB	2.38	59	0.661 ± 0.163	0.71	18.56	P < 0.001 (5)*
	MAR	1.77	56	0.714 ± 0.150	0.93	6.70	P < 0.005 (1)*
	APR	3.62	57	1.140 ± 0.269	1.34	137.8	P < 0.001 (9)*
	MAY	9.33	63	0.921 ± 0.181	1.76	167.7	P<0.001 (7)*

(1986: r = -0.098, N = 60; 1987: r = 0.086, N = 160; 1988: r = -0.010, N = 84).

Chi-square analyses indicated no significant pattern for the distribution of reproductive adults being alone, paired, or with three or more individuals on a given colony (X^2 = 4.41, P < 0.111, df = 2, N = 95). Of the sexually mature animals on shells, approximately equal numbers were found alone, paired, or with three or more nudibranchs. The > 3 category showed the lowest percentage (Fig. 3). On the other hand, non-reproductive nudibranchs showed a significant pattern of aggregation (X^2 = 92.88, P < 0.0001, df = 2, N = 768), with 49% of the animals occurring in groups of three or more on a colony. Significant differences existed in the three categories between the reproductive and non-reproductive individuals, suggesting behavioral differences between juvenile and adult nudibranchs (G-test, p < 0.001).

Size Frequencies

Mean size of *Cuthona nana* on colonies varied for each month over the 2.5 yr sampling period (Fig. 4). Mean size increased from July to October for both 1986 and 1987. In all three years, more non-reproductive than reproductive individuals were present each month (Fig. 5). Reproductive adults were present on colonies in all months for 1986 except July and November, and were absent in August, November, and December 1987 (Fig. 5). This does not mean that adults were absent from Gosport Harbor, but they were not present on the colonies collected. Adults were present on colonies in all five of the months sampled in 1988. The percentage of adults in the summer months varied for 1986 and 1987 and decreased in late summer and early fall, followed by an increase in October for both years.

Nudibranch movement on and off of colonies

The results of nudibranch movement on and off of *Hydractinia polyclina* colonies indicated that adults were more active; they averaged one to two moves on or off crabs with colonies per day (Fig. 6). These numbers are most likely underestimates, because more excursions from colonies could have occurred within the time of census. Adults (12-20 mm) were more active than juveniles in both trials; often during the adult trials, animals were observed

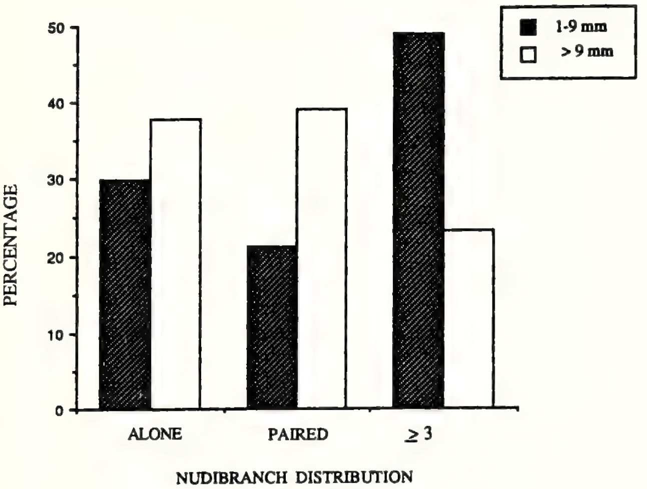

Fig. 3. The percentage of non-reproductive (1-9 mm) and reproductive (> 9 mm) *Cuthona nana* scored as solitary, paired, or with three or more individuals on a hydroid-covered hermit crab shell from May 1986 to May 1988. Chi-square tests indicated a significant difference among the three categories for non-reproductive animals ($P < 0.0001$, $df = 2$) and non-significant differences for reproductive individuals.

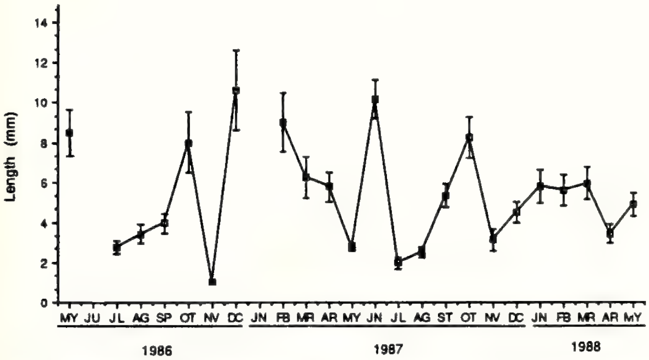

Fig. 4. Mean size of *Cuthona nana* on hydroid-covered hermit crab shells for each month of collection. The bar for each mean represents standard error.

mating and laying egg masses on rocks and the sides of the sea water tables. No egg masses were laid on colonies of *H. polyclina* during the experiment. Juvenile nudibranchs (2-4 mm) did not leave shells covered with *H. polyclina* under the experimental conditions employed (Fig. 6). No juvenile nudibranchs left the colonies where they were initially placed in any of the four trays.

Hermit crab mobility

Considerable hermit crab activity occurred in a 24-h period in view of the fact that this experiment was conducted during the colder months of the year. In 24-h, the mean number of crabs caught per pitfall trap ranged from 15-26 (Jan.: 15.4 ± 10.2 SD, N = 308; Feb.: 21.3 ± 11.0 SD, N = 383; Mar.: 15.0 ± 11.0 SD, N = 251; May: 26.7 ± 13.9 SD, N = 533). The mean number of crabs caught that were colonized with *H. polyclina* ranged from 3-8 (Fig. 7). On average, six crabs with *H. polyclina* passed a given area during a 24-h period. This supports the probability that crabs with hydroids were likely to pass by a given nudibranch within a 24-h period. The number of shells with (and without) *H. polyclina* increased from March to May. The four months sampled were during the time of year with low crab and hydroid densities, thus providing conservative estimates (Grant, 1963; Rivest, 1978).

DISCUSSION

Nudibranch population patterns

The population patterns of *Cuthona nana* at Gosport Harbor indicate a sub-annual life cycle during which the species undergoes several generations in a year (Miller, 1962; Thompson, 1964; Harris, 1973, 1975; Todd, 1981, 1983). The results of this study paralleled those of Rivest (1978) and Folino (1985) but also provided information on summer abundances during months when data had not been previously obtained. The presence of juveniles throughout all months sampled, in conjunction with adults present in the summer and fall and continuous egg-laying, indicates the existence of overlapping generations.

Although most species of nudibranchs with several generations per year feed on seasonal prey (Miller, 1962; Clark, 1975; Harris, 1973; Todd, 1981, 1983), two species, *Phestilla* sp. and *Cuthona nana*, do not (Harris, 1975; Rivest, 1978, and Folino, 1989, respectively). Prey availability for *C. nana* depends upon crab location; although most crabs migrate to deeper water during colder months, there are still crabs present in shallower water with colonies available for food during the winter (Rivest, 1978; Folino, 1989). Thus, the population of *C. nana* at Gosport Harbor is able to persist throughout the year due to the presence of crabs with colonies.

Partial predation

This nudibranch-hydroid association is an example of partial predation on colonial organisms, a phenomenon that has received increased attention in recent years for both plants and animals (Jackson, 1985; Coates and Jackson, 1985; Harper, 1985; Harvell and Suchanek, 1987; Todd and Havenhand, 1989). *Hydractinia polyclina* regenerates when damaged by predators (Christensen, 1967; Sutherland and Karlson, 1977; Karlson, 1978; Buss *et al.*, 1984; Folino, 1985; McFadden, 1986). Based on previous grazing rates (Folino, 1985, 1993), a large *Cuthona nana* could graze approximately one-quarter (23%) of an average-sized colony in less than a 24-h period and leave a substantial portion for continued growth. These estimates of

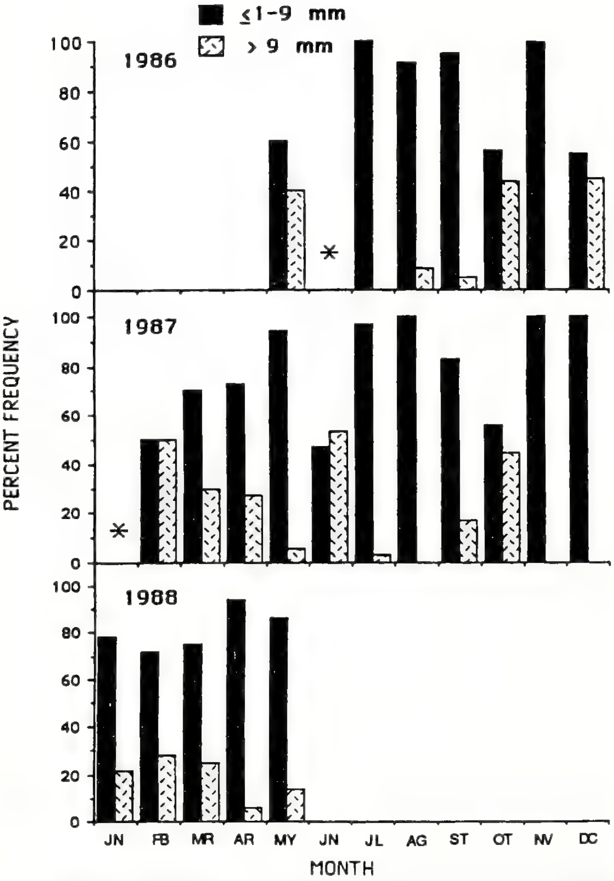

Fig. 5. Percent frequencies of non-reproductive (1-9 mm) and reproductive (> 9 mm) *Cuthona nana* collected from May 1986 to May 1988. (*, sampling not possible due to rough seas).

grazing consider only one large nudibranch on a colony; obviously two or more large animals would do more damage. Even so, grazing does not completely decimate the prey as a food source. Furthermore, the majority of colonies collected did not support nudibranchs; the percentage of colonies (or shells) with nudibranchs did not exceed 60% (Fig. 2). This again suggests that *C. nana* is not limited by prey availability.

Laboratory data on colony growth and regeneration are ambiguous. In small colonies, predator consumption rates (large *Cuthona nana* eat 200-500 polyps in a 24-h period at 12°C; Folino, 1993) can outstrip hydroid growth. However, growth rates also increase with temperature and colony size (McFadden *et al.*, 1984; Folino, 1985) suggesting that larger colonies produce polyps at a rate closer to that of polyp removal by predators.

The presence of chitinous spines on most colonies of *Hydractinia polyclina* in this study prevents complete removal of polyps by *Cuthona nana* (see Folino, 1993), a situation analogous to that seen in bryozoan zooids where spines reduce nudibranch grazing rates (Yoshioka, 1982; Harvell, 1984). Polyps that have been partially eaten can clearly regenerate (Folino, 1993). Therefore, *C. nana* do not decimate prey as is true in other nudibranch-hydroid associations (Clark, 1975; Todd, 1981, 1983).

Nudibranch distributions and movement

The aggregation of sexually immature *Cuthona nana* on *Hydractinia polyclina* (and lack of aggregation of reproductive individuals) differs from studies of other species in which aggregation occurs in both sexually mature and immature individuals (Miller, 1962; Clark, 1975; Todd, 1981, 1983). Potts' (1970) work with *Onchidoris bilamellata* (Linné, 1767) [= *O. fusca* (Müller, 1776)] suggested that the nudibranchs probably remain on the rock where they initially settled because ample food and mates are available. Field observations by Todd (1978a, 1979) for *O. muricata* (Müller, 1776) and *O. bilamellata* showed increased aggregation during the breeding season, suggesting that animals stay within an area where food and other sexually mature individuals are present. The distribution pattern seen in *C. nana* populations could be produced by the behavior of the nudibranchs. As juveniles, they hatch from eggs laid on rocks, mussel shells, and *Chondrus* (Rivest, 1978; Folino, 1993). Juveniles appear to be picked up by *H. polyclina* gastrozooids that sweep along the ocean bottom, and they remain on the colony until sexually mature. Adults leave hydroid colonies and follow mucus trails to find potential mates, as do other mollusks (Lowe and Turner, 1976; Todd, 1978b, 1979; Gerhart, 1986; see review: Hadfield and Switzer-Dunlap, 1984). During the peak reproductive periods for *C. nana* from April through September, Rivest (1978) and Folino (1989)

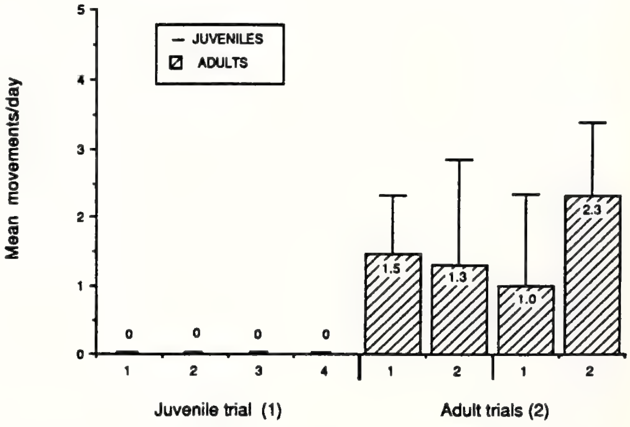

Fig. 6. Nudibranch movement on and off of *Hydractinia* colonies. One trial with four replicates (left) indicating that juvenile *Cuthona nana* (2-4 mm) did not leave colonies during a 21-d period. Alternatively, adults (12-20 mm) moved on and off an average of 1-2 times per day (two 12-d trials, right). The bar for each mean represents standard deviation.

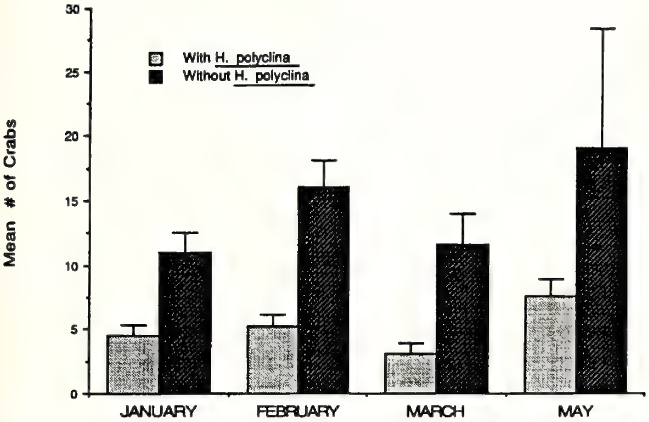

Fig. 7. Mean number of hermit crabs caught in pitfall traps in a 24-h period during four months in 1988. Twenty traps were sampled in January and May while 18 and 17 traps were sampled in February and March, respectively. The bar for each mean represents standard deviation.

observed adult *C. nana* more frequently crawling on the bottom of Gosport Harbor. Mating does occur on the hydroid colonies, but occurs more often off of them (Harris *et al.*, 1975; Rivest, 1978; Folino, 1985, 1989). The results of the laboratory nudibranch movement experiment support these field observations of nudibranch behavior (Fig. 6). *C. nana* behavior differs from those of the dorid nudibranchs, *Onchidoris* spp., where encounters with mates are enhanced due to aggregation near stationary prey (Potts, 1970; Todd, 1978a, 1979). Movement of adult *C. nana* off of its mobile prey increases the chances of encountering mates.

The life history of *Cuthona nana* differs from those of other hydroid-feeding aeolids. In most species, larvae are planktonic, and settle on sessile prey for growth through sexual reproduction (Todd, 1981, 1983). In contrast, *C. nana* at Gosport Harbor lacks a planktonic veliger and exploits a mobile prey. Once picked up by a passing crab, juveniles feed on basal mat tissue until they reach ca. 5-6 mm, when they are large enough to consume polyps (Folino, 1993). Furthermore, crab mobility could help distribute juvenile nudibranchs over several colonies preventing over-predation of some (especially small) colonies (Rivest, 1978). This decreases the degree of grazing on an individual colony (Folino, 1993) and could also promote genetic variation in the population by 'mixing up' cohorts.

Cuthona nana behavior is similar to that of plant bugs (Miridae) (Price, 1980). Adult plant bugs are large ectoparasites and are mobile, whereas the immature stages spend all of their time on a single host. Juvenile *C. nana* showed a similar behavior and did not switch colonies in the laboratory. Thus differences exist between adult and juvenile residence time, which affect the degree of grazing on the prey. This behavior in *C. nana* seems to parallel the prudent parasite model because partial (rather than total) consumption of the prey is important to the predator's survival (Holmes, 1983).

Hermit crab movement

Cuthona nana at Gosport Harbor do not lay egg masses on colonies of *Hydractinia polyclina*, but rather on the ocean floor. Because hermit crab movement will bring prey to juveniles on the bottom, adult *C. nana* at Gosport Harbor do not jeopardize the juveniles' probability of finding food by depositing egg masses off of the colonies (Rivest, 1978; Folino, 1993). Non-planktonic development in the *C. nana* population at Gosport Harbor could actually be an adaptation to a mobile prey and to trophic stability (Clark and Goetzfried, 1978). With yolk present at metamorphosis, juveniles can survive for up to ten weeks without feeding at 4°C (Rivest, 1978), which is ample time for a crab to bring food (Fig. 7). The results of the pitfall experiment indicate that crabs with colonies of *H. polyclina* are fairly active over a 24-h period and provide sufficient opportunities for prey encounters. This experiment in conjunction with the monthly collections of shells with colonies indicates a non-seasonal food supply for *C. nana. Hydractinia* is continuously available in Gosport Harbor, especially at the time of metamorphosis, allowing for non-planktonic development.

There are several similarities between the life history of *Cuthona nana* and that of a parasite (Price, 1980; Strand and Obrycki, 1996); such similarities shed insight on the maintenance of *C. nana*'s population. *C. nana* is much like a parasite in being a specialist on *Hydractinina polyclina*. The phenomenon of juveniles being picked up by their prey is similar to a host-parasite relationship, such as is seen with intermediate stages of parasitic trematodes, flukes, or hookworm larvae (Cheng, 1970). Juvenile *C. nana* ("parasites") on the ocean floor are picked up by prey ("host") passing by; alternatively adults can conceivably crawl onto a colony while a crab is temporarily stationary and is filter feeding (Gerlach *et al.*, 1976; Rivest, 1978; pers obs.). Furthermore, similar to a parasite (Price, 1980), *C. nana* is not a fast-moving predator "chasing" mobile colonies of *H. polyclina*. Adult *C. nana* can crawl onto a colony while a crab is stationary or onto a colony without a crab in the shell. Thus, the movement and egg-laying behavior of *C. nana* adults at Gosport Harbor along with the hydroids' ability to regenerate and the mobility of the prey on the crab shells all contribute to the persistence of prey availability throughout the year. Prey availability is very important in determining the population patterns of a specialized predator like *C. nana*. These factors allow for the sub-annual life cycle and perhaps non-planktonic development of *C. nana* with prey not being the limiting factor for the population ecology of this prey-specific nudibranch.

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Temporal and spatial patterns of abundance in the gastropod assemblage of a macrophyte bed

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Abstract: Although gastropods are more common and diverse in macrophyte beds than in areas without littoral vegetation, little is known of fine-scale patterns of abundance within macrophyte beds, or changes in abundance of species through time. I present data here on such patterns in the gastropod assemblage found in Carrol Lake, Wisconsin. The assemblage was quite diverse, with 13 species, four of which had mean densities greater than 100/m^2. The assemblage was dominated by small, thick-shelled species, especially *Amnicola limosa* Say, 1817. Gastropod density decreased across two field seasons, although trends within each field season were for increases with time in the abundance of most species, especially in shallower habitats. At the micro-habitat scale, abundances of the most common species were positively correlated, perhaps because they prefer to colonize the same macrophytes, based on the results of laboratory macrophyte-choice experiments.

Key words: gastropods, micro-distributions, macrophyte choice

Gastropods are clearly more abundant and diverse in macrophyte beds than in littoral-zone habitats without vegetative cover (Brown *et al.*, 1988; Brown, 1991; Brown and Lodge, 1993). However, few studies have considered fine-scale patterns in the abundance of gastropod assemblages within macrophyte beds, or how such patterns vary through time or space. In an earlier paper (Brown and Lodge, 1993), we studied whether gastropod distributions at the macrohabitat (*e. g.* sand versus cobble versus macrophytes) scale were determined by habitat choice. Specifically, we worked experimentally with several gastropod species common in northern Wisconsin lakes, and found that most species, on a surface-area specific basis, preferred cobble over macrophyte substrata. However, given the fact that total abundances and diversity of gastropods are still greater in macrophyte beds because of the greater substratum surface area per unit bottom area, I consider it still important to look at patterns of microdistribution and habitat choice within macrophyte beds.

Here I present such data describing temporal and depth-related trends in the abundance of a gastropod assemblage in a macrophyte bed in a northern Wisconsin lake. I present data on changes in the abundances of gastropods over two seasons, and describe how abundances change through time within each season at different depths. I am also interested in whether gastropods show similar use of space at the micro-habitat (*e. g.* single sample) level. Finally, I test for differences in macrophyte choice, with several gastropod species, on several macrophytes common in northern Wisconsin lakes. The purpose of these experiments was to determine if the high levels of overlap of species at the micro-habitat level (see results) could be explained by similar patterns of macrophyte use.

METHODS

SAMPLING OF GASTROPODS

Sampling was conducted in the near-shore littoral zone of Carrol Lake, a circum-neutral, mesotrophic lake in Vilas County, Wisconsin (for a general description of these lacustrine habitats, see Lodge *et al.*, 1994). Temperatures ranged from 2-3°C in winter to a maximum of 20°C in late summer. Dissolved oxygen readings ranged from 10 mg/l in summer to 5 mg/l in mid-winter, underneath the ice. Substrata changed from sand and cobble in the first few meters from shore to sparse macrophytes, and then a dense macrophyte bed at depths greater than 0.5 m (Fig. 1). Filamentous green algae were common on the cobble and sand, and macrophyte diversity increased with distance from the shoreline. The most common macrophyte species were *Vallisneria americana* Michaux, *Elodea canadensis* Michaux, *Potamogeton robinsii* Oakes, *Sagittaria* spp., *Najas flexilis* Rostkovias and Schmidt, *Ceratophyllum*

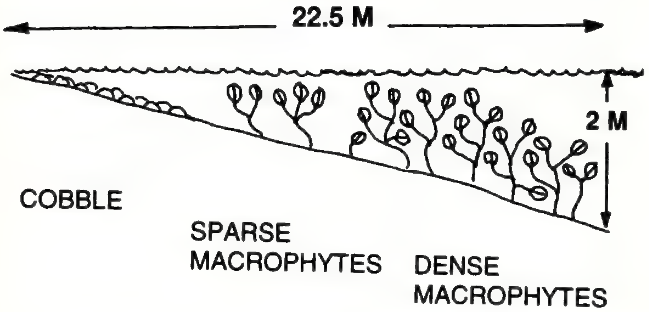

Fig. 1. Transect of the sampling site, indicating depth, substratum type, and macrophyte density.

demersum Sieber ex Chamisso, *Megalodonta beckii* Greene, *Myriophyllum exalbescens* Fernald, *P. amplifolius* Tuckerman, and *P. richardsonii* Rydberg.

A 500 m^2 section of littoral zone (approximately 22.5 m along the shoreline and 22.5 m out into the lake) was marked off along the northeastern shoreline of Carrol Lake, and all sampling was conducted within this area. The site was sampled over two field seasons in 1990 and 1991. In each season, five sampling groups were completed. The first sampling group was within two weeks of ice-out (usually in mid-May), and each successive sampling group was taken at approximate three-week intervals through the summer, with final sampling in late September. Each sampling group consisted of eight benthic cores, stratified into three depth bands based on relative area within the 500 m^2 area: inshore (less than 0.5 m depth, two samples), mid-depth (0.5-1 m depth, three samples), and deep (greater than 1 m depth, three samples). The 500 m^2 area was divided into 3 x 3 m plots, and these 9 m^2 plots were chosen randomly within each stratum at each date for sampling.

A 0.3 m-long benthic corer, constructed of 15 cm-diameter PVC pipe (sampling area = 182 cm^2), was inserted by two SCUBA divers 3 cm into the substratum so that a horizontal slit in the side of the corer was at least 1 cm below the sediment surface, and an aluminum restraining plate was inserted. The top 1 cm of sediment, overlaying macrophytes, and any snails in the sediments or macrophytes were then collected in the corer. A large plastic bag was held over the corer as it was brought to the surface to ensure that macrophytes or gastropods were not lost from the sample. More details on the corer and basic sampling methods can be found in Klosiewski (1991) and Lodge *et al.* (1994) .

Samples were processed through a series of sieves (ranging from 2 mm to 0.5 mm mesh) to remove fine sediments but retain snails, which all have a minimum diameter greater than 0.5 mm (Lodge *et al.*, 1987, 1994). Samples were then sorted in shallow enamel trays with the aid of a magnifying lens and light (Brown, 1991). Macrophytes were removed and vigorously shaken in a water-filled tub to dislodge gastropods (preliminary experiments revealed this technique increased snail recovery and minimized time spent sorting).

Gastropod abundances were converted to a per m^2 basis, and both total gastropod abundance and the abundances of the each of the five most common gastropods were analyzed in a three-way analysis of variance (two years times five sampling groups times three depth strata). I considered year, sampling group, and depth to be fixed effects. Abundances were log-transformed before analysis of variance because of mean-variance correlations, but raw data are portrayed in all figures. Thus, I could look for seasonal, yearly, or depth-related trends in the abundance of the whole gastropod assemblage, along with differences among the distributions of each of the five most common species.

To assess more fine-grained differences in the distributions of the gastropods, I also looked for correlations in the abundance of the snails on a per sample basis. This is essentially equivalent to calculating niche-overlap values for each of the species pairs, because most niche-overlap metrics (for example, the traditional Levins-MacArthur overlap statistic) are similar to correlation coefficients (Brown, 1982). The Pearson product-moment correlations were calculated from data pooled over both years, all sampling groups, and depths.

MACROPHYTE COLONIZATION EXPERIMENT

This experiment was conducted indoors at the Trout Lake Biological Station of the University of Wisconsin in July 1987. The experimental units were circular plastic tubs, 50 cm in diameter and 15 cm deep, with a sand substratum of 2 cm and a water depth of 12 cm. Water temperature was 20°C and lighting was on a 14L:10D schedule. Each pan had six stems each of four macrophytes: *Ceratophyllum demersum*, *Elodea canadensis*, *Potamogeton robinsii*, and *P. richardsonii*.

These macrophyte species were chosen both because they are common species in northern Wisconsin lakes (Lodge *et al.*, 1994), and because they vary in morphology, ranging from thin-leaved (*Ceratophyllum* and *Elodea*) to relatively broad-leaved (both *Potamogeton* spp.). The wet masses of the individual stems were chosen so that surface area was standardized among the four species at 64 cm^2. These masses were 0.71 g (*Elodea*), 1.2 g (*Ceratophyllum*), 0.85 g (*P. robinsii*), and 0.93 g (*P. richardsonii*). Standardization of surface area was necessary because colonization rates are dependent on surface area (Kershner and Lodge, 1990). Stems were hap-hazardly inserted in the sand substratum, and weighed down with lead strips so that stems were vertical.

Fifty snails of each of four gastropod species were introduced in mono-specific populations to the tubs. Numbers of snails colonizing each macrophyte were recorded after 8 hr because preliminary experiments indicated colonization peaked by that interval. Four species were chosen either because they were abundant in Carrol Lake (*Amnicola limosa*, Say 1817; *Physa gyrina* Say, 1821; *Helisoma anceps* Menke, 1830), or to represent the range of pulmonate families common in these Wisconsin lakes (*Lymnaea emarginata* Say, 1821). There were five replicates for each gastropod species. Statistical analysis of the data was as a two-way analysis of variance (four snail species times four macrophyte species), with a split-plot arrangement of treatments (macrophyte species were present as sub-plot variables in tubs with each gastropod species).

RESULTS

GASTROPOD SAMPLING

The gastropod assemblage of Carrol Lake was quite diverse and abundant (Fig. 2), with 13 species total. Four species had mean abundances (over all sampling groups in both years) greater than 100 individuals per m^2: *Amnicola limosa* (a caenogastropod), *Valvata tricarinata* (Say, 1817) (a "lower heterobranch"), and *Gyraulus parvus* (Say, 1817) and *Physa gyrina* (both pulmonates). Four additional pulmonates had intermediate levels of abundance (greater than $5/m^2$): *Helisoma anceps, H. companulatum* (Say, 1821), *G. hirsutis* (Say, 1817), and *Promenetus exacuous* (Say, 1821), along with the caenogastropod *Campeloma decisum* (Say, 1816). Four additional species were rare (*e. g.* collected in fewer than five of the sampling groups): three pulmonates [*Helisoma trivolvis* (Say, 1817), *Fossaria galbana* (Say, 1825), and *Laevapex fuscus* (Adams, 1841)], and one caenogastropod [*Viviparus georgianus* (Lea, 1834)]. Most of the common gastropods were fairly small (Fig. 2), with the six most common species averaging (again over all sampling groups) less than 10 mm in shell length (spiral-shelled species) or diameter (plano-spiral species).

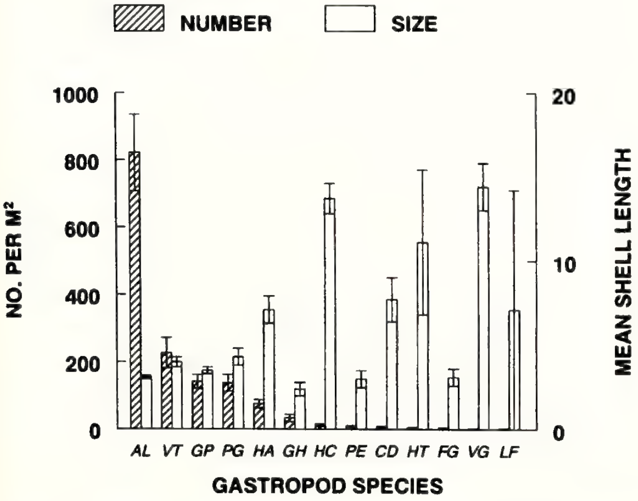

Fig. 2. Abundance of gastropod species, averaged over all sampling groups, years, and depth strata, ± standard errors, along with mean shell lengths ± standard errors. See Table 1 for the acronyms of the five most common species. (CD, *Campeloma decisum*; FG, *Fossaria galbana*; GH, *Gyraulus hirsutis*; HC, *Helisoma companulatum*; HT, *H. trivolvis*; LF, *Laevapex fuscus*; PE, *Promenetus exacuous*; VG, *Viviparus georgianus*).

Table 1. Results of a three-way analysis of variance on total abundance of gastropods (TOTDEN), and the abundance of the five most common species. Values are F statistics. There were 44 error degrees of freedom, one degree of freedom for year, four degrees of freedom for sampling group, and two degrees of freedom for depth. (AL, *Amnicola limosa*; GP, *Gyraulus parvus*; HA, *Helisoma anceps*; PG, *Physa gyrina*; VT, *Valvata tricarinata*; *, $P < 0.05$; **, $P < 0.01$).

EFFECT	TOTDEN	AL	VT	GP	PG	HA
Year	4.5*	9.2**	0.2	0.4	.01	1.4
Sampling Group	6.4**	6.0**	6.7**	0.1	0.4	2.2
Yr x Group	1.7	0.2	0.8	0.7	2.1	0.6
Depth	8.1**	9.0**	2.7	0.2	4.0*	4.9*
Yr x Depth	0.6	0.3	0.4	1.9	0.7	0.2
Group x Depth	3.0*	3.1*	2.8*	0.9	0.4	1.0
3-way Interaction	0.5	0.7	0.4	0.8	0.8	0.5

There were complicated changes in the total abundance of gastropods across years, sampling groups and depths (Table 1; Fig. 3). The significant year-effect in the analysis of variance was because of a decrease in gastropod abundance over all sampling groups and depths in 1991. The significant sampling-group effect was because of an increase in abundance of most species later in the field season in both years. There was also a significant trend toward increased gastropod density and diversity in deeper strata. For example, the mean number of species per sample, over both years, was 4.3 for the shallow substratum, 6.3 at intermediate depths, and 6.4 at the deepest depths. Finally, there was a significant interaction between sampling group and depth for total gastropod density. The basic trend appears to be a greater rate of increase in numbers in the shallower depths with time in each season.

The pattern of significance of the F-values in the analysis of variance for *Amnicola limosa*, the most abundant species, was the same as for total gastropod density. This probably indicates that changes in the dynamics of this species are driving the overall effects (Table 1; Fig. 4). Again, densities decreased in 1991 (mean density was lower in 13 of the 15 sampling group and depth combina-

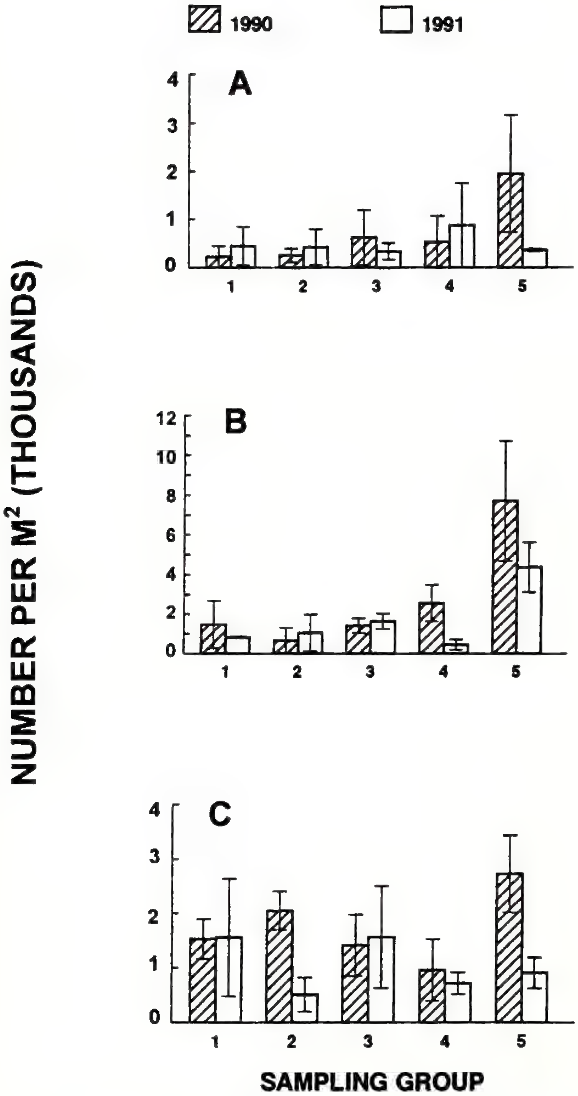

Fig. 3. Total abundance of all gastropod species ± standard errors in five sampling groups in two years, for (A) shallow, (B) intermediate, (C) deep depth strata.

tions [Fig. 4], with a mean reduction in density of 60%). There was also a trend within both years for increased abundances later in the season, with density (averaged over both years and all depth strata) increasing from 473/m^2 in the first sampling group to 1,765/m^2 in the last sampling group.

There was also a significant change in abundance with depth for *Amnicola limosa* (over both years and all sampling groups); densities increased from 388/m^2 in the shallow stratum to a maximum of 1,360/m^2 at intermediate depths, and dropped to 728/m^2 in the deep substratum. Finally, there was a greater increase in abundance through time in the shallow and intermediate areas, while densities were more stable in the deep stratum, explaining the significant sampling group by time interaction (Table 1). For example, densities, averaged over both years, increased from 170 /m^2 in the first sampling group to 622/m^2 in the last in the shallow stratum, and from 523 to 3,748/m^2 in the intermediate stratum, but were quite constant in the deep stratum (starting density of 928/m^2 and ending density of 924/m^2).

For *Valvata tricarinata*, only the sampling-group main effect and the sampling group x depth interaction were significant. Averaged over both years and all depth

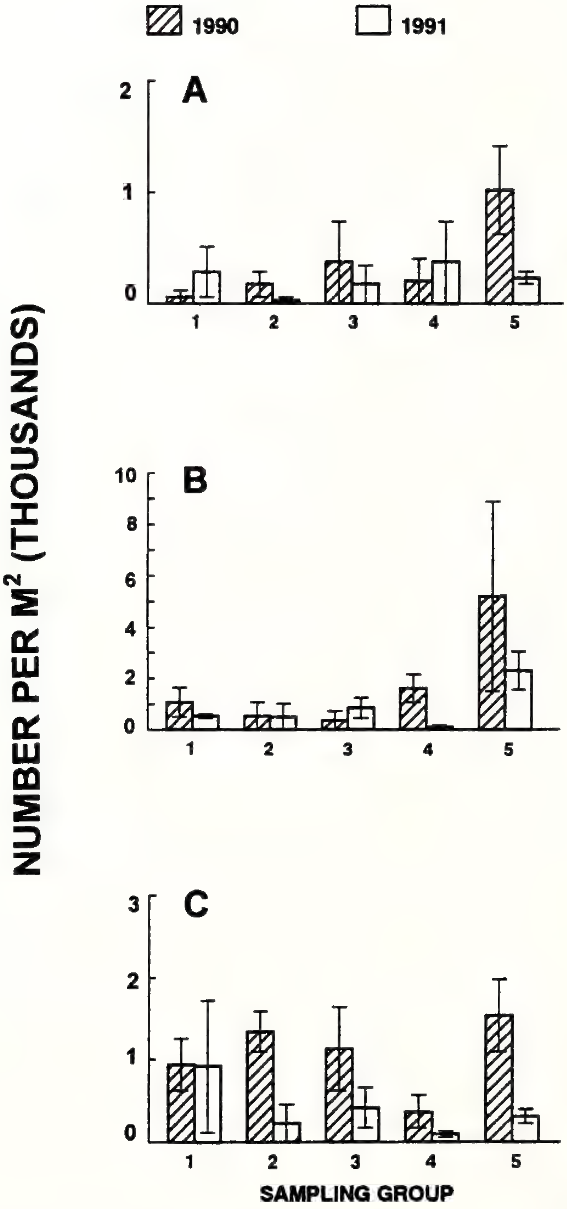

Fig. 4. Abundance of *Amnicola limosa* ± standard errors in five sampling groups in two years, for (A) shallow, (B) intermediate, (C) deep depth strata.

substrata, densities increased from $96/m^2$ in the first sampling group to $661/m^2$ in the fifth sampling group. The significant sampling group x depth interaction probably occurred because densities increased (averaged over both years) from 0 to $354/m^2$ in the shallow stratum from the first to last sampling group, and from 85 to $1,347/m^2$ at intermediate depths. However, they were again fairly constant in the deepest stratum, changing from only 207 to $283/m^2$.

For the remaining three species that had fairly high densities overall (*Gyraulus parvus*, *Physa gyrina*, and *Helisoma anceps*), there were no differences in density between years, or across sampling groups (Table 1). However, there was a significant depth effect for the latter two species. For *P. gyrina*, densities (averaged over all dates and both years) increased from 37 to $207/m^2$. For *H. anceps*, densities actually peaked ($127/m^2$) at the intermediate depth compared to the shallow ($22/m^2$) and deep habitats ($48/m^2$).

At the level of the individual sample, there were significant positive correlations in the abundance of species (Table 2). The abundance of the most common species, *Amnicola limosa*, was positively correlated with each of the four other fairly common species. The abundance of *Valvata tricarinata* was positively correlated with *Physa gyrina* and *Helisoma anceps*. None of the other correlation coefficients were significant. Thus, on a per sample basis, "hot spots" for snail abundance existed, and many species co-occurred at these sites.

MACROPHYTE COLONIZATION EXPERIMENT

The results of this experiment clearly indicate that all four species of gastropod have the same rank-order of macrophyte preference (Fig. 5). The plant main effect was highly significant ($F_{3,79} = 43.8$, $P < 0.0001$), and the plant-snail interaction was not significant ($F_{9,79} = 2.0$, $P = 0.07$), indicating the preference rank was similar in each of the snail species. However, the snail main effect was also highly significant ($F_{3,12} = 13.2$, $P = 0.0004$), indicating differences in colonization rates among snails. *Ceratophyllum demersum* had the lowest colonization rates, averaging 1.6 snails per plant (over all snail species). Next came *Elodea canadensis* with 3.5 snails per plant, *Potamogeton robinsii* with 8.0 snails per plant, and finally *Potamogeton richardsonii* with 12.6 snails per plant. Most gastropods had fairly similar colonization rates, except *Lymnaea emarginata* which had the lowest colonization rates on each macrophyte. This is not surprising, because this species is common on periphyton-covered cobble in shallow water in Wisconsin lakes, not on macrophytes (Weber and Lodge, 1990; Brown and Lodge, 1993). In summary, most gastropods had fairly similar colonization rates on each of the macrophytes tested, and colonized broad-leaved *Potamogeton* species more readily than thinner-leaved species like *Elodea* or *Ceratophyllum*.

Table 2. Pearson product-moment correlations among the abundances of the five most common gastropods in the samples. See Table 1 for species acronyms and symbols for significance.

	AL	VT	GP	PG	HA
AL	1	0.65**	0.25*	0.37**	0.51**
VT		1	-0.02	0.29*	0.37**
GP			1	0.17	0.09
PG				1	0.19
HA					1

DISCUSSION

SPATIAL OVERLAP

The gastropod assemblage of Carrol Lake was quite diverse, with 13 species total, 5-6 of which had fairly high abundances. This is a fairly representative sample of the species present on macrophytes in northern Wisconsin lakes, and is certainly not an exceptionally diverse assem-

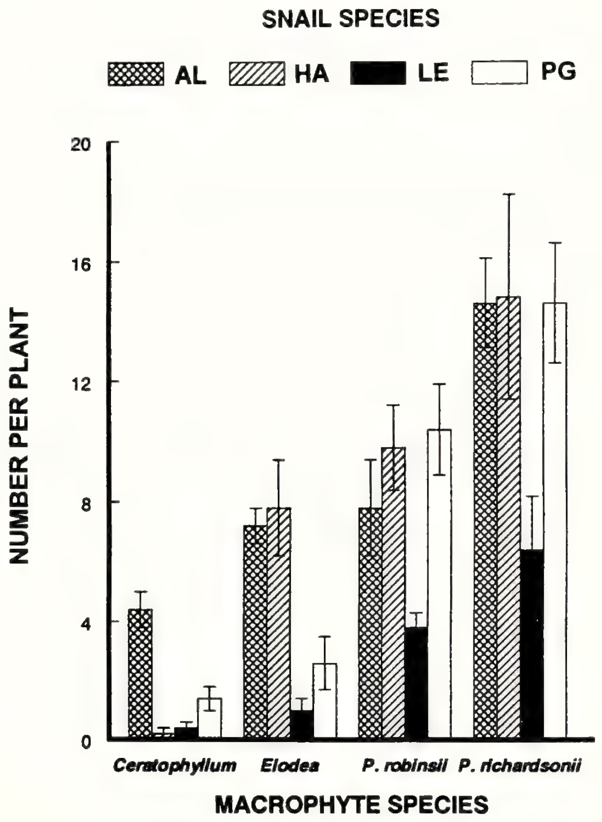

Fig. 5. Mean numbers of four gastropod species colonizing four macrophyte species, ± standard errors. (LE, *Lymnaea emarginata*; see Table 1 for other acronyms).

blage for the area (Brown and Lodge, 1993). The gastropod assemblage was dominated by small, thick-shelled species like *Amnicola* and *Valvata*. Larger species with thick shells, like *Helisoma* spp., *Viviparus georgianus*, and *Campeloma decisum* were present, but rare. The only relatively thin-shelled species (Stein *et al.*, 1984) that attained higher abundances were *Gyraulus parvus* and *Physa gyrina*. The higher relative abundances of small, thick-shelled species could be the result of selective predation by fish, which tend to remove larger, thinner-shelled gastropods (Stein *et al.*, 1984; Osenberg and Mittelbach, 1989; Klosiewski, 1991).

The reduced gastropod abundances in the second year are unexplained, but could indicate significant yearly variation in abundances caused by some abiotic factor (harsh winter weather, etc.). I do not believe the reduced abundances were caused by sampling disturbance, because the area sampled per season was only 0.2 % of the 500 m^2. However, seasonal increases in snail densities in late summer are fairly easy to explain. Most of these gastropods have a spring and early-summer recruitment period, resulting in population increases by the end of the summer (Brown, 1991). The increased densities with depth were probably the result of increased macrophyte biomass at the intermediate and deep strata, because macrophyte density is positively correlated with gastropod density and diversity (Brown and Lodge, 1993). The constancy of numbers through time in the deeper areas, and the increases in shallow areas as the season progressed could be caused by annual migrations. Cheatum (1934) was the first to document movements of snails to deeper water in winter, and back to shallower water in spring, and several others have noticed the same trend (Clampitt, 1974; Boag, 1981). However, an alternative hypothesis would be that snail populations in shallower areas reproduce earlier and thus grow more rapidly because of higher water temperatures (Brown, 1991).

Amnicola limosa was the most abundant species, and it is therefore not surprising that the same treatment effects were significant for this species and gastropod abundance as a whole. Again, densities built up into the field season, were lower in the second year, and increased more with time in shallow areas. For the species in the second tier of abundance, fewer treatment effects could be detected. This could simply be because of their lower abundances and thus patchy distributions (Brown, 1991). The trend for increased abundance with depth, however, occurred for most of the abundant species, again indicating the importance of macrophyte cover to snail abundance.

OVERLAP ON MACROPHYTES

At the level of the individual sample, the most common species had positive correlations among themselves in abundance. This is probably because of the patchy distribution of snails in general: certain sites had higher snail densities of all species, probably because of variation in macrophyte diversity and cover, etc., among samples. For example, certain species of macrophytes might have greater periphyton abundances or periphyton species of higher nutrient quality, differ in their accessibility to snail grazers, or offer more of a refuge from predation. All of these hypotheses deserve further study. These data do indicate, however, that there is little spatial partitioning at the scale of the microhabitat among these snail species.

In fact, the laboratory experiment suggests that there is almost a remarkable degree of similarity in colonization rates of a group of macrophytes among the four species of snails studied. The snails all prefer broader-leaved species like *Potamogeton* over thin-leaved species like *Elodea* or *Ceratophyllum*. Several mechanisms could produce such results. Either broader-leaved species are colonized by a richer periphyton assemblage, or broader-leaved species could have growth forms more suitable for colonization, or broader-leaved species could provide more of a refuge from visually orienting predators. The first and last hypotheses remain unexplored, but the second hypothesis contradicts a laboratory study by Kershner and Lodge (1990). Kershner and Lodge found higher colonization rates by two snail species on more finely divided artificial macrophytes (*e. g.* that would mimic thinner-leaved species like *Elodea* or *Ceratophyllum*), but did conclude that the particular relationship of macrophyte growth form to colonization rate depended on the animal group under study. Whatever the underlaying mechanism, the data suggest there is little potential for differential use of macrophytes to be a mechanism of niche partitioning by snail species. In fact, the similar patterns of macrophyte choice among species could explain why there were positive correlations among the abundances of the most common species in the sampling data.

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Sympatric speciation of freshwater mussels (Bivalvia: Unionoidea): a model

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Abstract: Speciation of freshwater mussels (Bivalvia: Unionoidea) can occur sympatrically (ecologically) via new glochidial host acquisition. Due to the characteristics of the unionoidean life cycle, this hypothesis overcomes the objections of the classical allopatric speciation paradigm, namely homogamy and linkage of mate and habitat preferences. Examples of freshwater mussel populations in various stages of the speciation process are provided from the literature.

Key words: Unionoidea, host race, parasitism, sympatric speciation, speciation model

Inclusion of a parasitic stage in the life cycle of a taxon influences the nature and rate of its speciation. Parasitic taxa can diverge in allopatry when peripheral populations of parasite and host co-evolve subsequent to isolation from their respective parent stocks (Mayr, 1970). Another mechanism of population subdivision available to parasitic species is the formation of host races. Although confusion exists regarding the meaning of the term "host race" (see Mayr, 1970), Bush (1974:3) defined it to describe "... an infraspecific category generally applied to populations of a parasitic species which exhibit distinct genetically-based preferences [for certain hosts]." The concept of host race formation has not, in the strict sense, been applied to freshwater mussels (Bivalvia: Unionoidea).

The impact of their glochidial hosts on the population structure of mussel species has been noted in the literature as far back as Ortmann (1920). Kat (1983; also Kat and Davis, 1984), for example, determined that unionoideans that utilized anadromous hosts maintained a high degree of genetic similarity between widely separated demes, while subpopulations of mussels that infested territorial fish diverged more rapidly. Because the population structure of freshwater mussels is so dependent on the ecology of their hosts, Kat (1984) also suggested that an intraspecific change in host fish might precede the formation of new species. Kat cited Bush's (1974) model of fruit fly speciation via host race formation as relevant to the Unionoidea, and I propose here to extend these ideas, in particular, their applicability to the concept of speciation without geographic isolation.

THE LIFE CYCLE OF FRESHWATER MUSSELS

Although the mechanics of the unionoidean life cycle have been sufficiently detailed elsewhere (*e. g.* Coker *et al.*, 1921; Kat, 1984), the biological implications of the various stages of this process have been largely ignored. To this end, an overview of mussel reproduction follows to emphasize certain points bearing upon the speciation model. The life cycle is here divided into four stages: spawning, brooding, encystment and dispersal, and adulthood.

Spawning

Male mussels expel their sperm directly to the water, and these are eventually entrained in the respiratory current of the female. Work with sea urchins (Pennington, 1985) has shown that even under low flow conditions, sperm diffuse rapidly, and the probability of fertilization decreases to nearly zero for females only a meter downstream. Unfortunately, such work has not been done for freshwater bivalves, but it is reasonable to expect a similar spatial-probability picture (Downing *et al.*, 1993). The range of fertilization, though, could be slightly increased by the mussels' greater ability to filter the water column and by the way they package their sperm (D. Ó Foighil, pers. comm.). Freshwater mussels, like oysters, possess spermatozeugmata (Edgar, 1965; Lynn, 1994) which deliver many sperm together rather than let them freely diffuse and be lost (see Ó Foighil, 1989).

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Brooding

Mussel embryos develop into larvae, known as glochidia, within the female's ctenidial marsupia. With maturity and appropriate environmental conditions, the glochidia are shed into the water. The extrinsic stimuli leading to glochidial release vary from seasonal fluctuations in temperature and food availability (as in *Margaritifera* spp.; Ziuganov *et al.*, 1994) to the presence of a potential host (as in *Lampsilis* spp.; Kraemer, 1970). There is intra- and interspecific variability in the timing of spawning, length of the brood period, and the time of glochidial release (*e. g.* Zale and Neves, 1982; Neves and Widlak, 1988).

Encystment and Dispersal

Glochidia undergo metamorphosis while encysted in the gill or fin epithelium of an appropriate fish (or, in one case, amphibian) host. Glochidia, incapable of selecting a suitable fish, reach their hosts passively and clamp to any tissue they contact (Lefevre and Curtis, 1910). Certain species, especially those of the genus *Lampsilis*, exhibit behaviors and morphologies capable of attracting fishes (Kat, 1984), increasing the probability of glochidia contacting a potential host. However, the actual specificity of attraction by mantle flaps and conglutinates has yet to be determined. After encysting to a potential host, those glochidia that do not evoke an immune response from the fish are able to complete their metamorphosis (Bauer and Vogel, 1987).

During encystment (lasting days to months), the glochidia are dispersed by the fish to new sites. Adult mussels are capable of limited movement, but the distances traveled are generally short (Amyot and Downing, 1997) and the progress is erratic (figured in Baker, 1928, and Mathiak, 1979); significant dispersal is facilitated only by their hosts. Because the habitat of an adult mussel is a function of where it excysted (Isley, 1911, 1914), it is expected to be found in the habitat preferred by its host. Mussels themselves have low habitat specificity (Strayer, 1981; Strayer and Ralley, 1993) and possess a plastic phenotype (*e. g.* Ortmann's Law of Stream Distribution; Ortmann, 1920) enabling their adaptability to various stream conditions (Watters, 1994b).

THE MODEL

Some model elements are common to all Unionoidea, such as limited fertilization range and conformation to the host habitat. Other factors which might not apply to all mussel species need to be evoked for the model presented here.

The model mussel population has high host specificity. Initially, this specificity is limited to one or possibly a few of the fish species present over the range of the mussel population. Although certain mussel species are characterized by low host specificity, other unionoideans do possess this type of high specificity (Hoggarth, 1992; Watters, 1994a).

Host specificity is under genetic control, and through spontaneous mutation the mussel population has developed the ability to parasitize a new host. The basis of host specificity is immunological (Bauer and Vogel, 1987). If the immune defenses of the infested fish recognize the surficial molecules of the glochidium as foreign, the parasite will be sloughed off. Such glochidial molecules must be under genetic control. That the host specificity of a particular mussel is under genetic control is also evidenced by the fact that mussels sharing a gene pool (*i. e.* species) tend to share host species.

Congeners often employ different host species (Hoggarth, 1992). During the course of their divergence from the ancestral population, additional hosts must have been added to the repertoire of these species. The mechanism by which this new host is added to the population need not necessarily be mutation; any of the normal processes that increase the genetic diversity of a breeding population (*i. e.* recombination, hybridization, etc.) are also acceptable.

The original fish host and the new host have different habitat requirements, and these fish have a strong preference for these habitats. For example, one host prefers riffles, while the other prefers pools; or one might prefer lakes and the other rivers. This can most often be achieved when the new and old hosts belong to different genera. The fish are not ecologically excluded from moving through habitats other than their preferred one.

The two fish hosts differ in their seasonal presence over the range of the mussel population. For instance, one host might be more prevalent late in the mussels' breeding season while the other fish might occur in greater numbers early in the breeding season.

The timing of glochidial release is heritable; it is ultimately under genetic control and can be acted upon by selection. Glochidial release (and spawning) can be triggered by an array of environmental cues such as day length, water temperature, and perhaps host presence. However, the basis for the mussels' perception and recognition of these cues is doubtless the result of physiological characters encoded in the mollusks' genome. Different mussel species exhibit different characteristic breeding periods

(Watters, 1994a), and one reasonable explanation is that the timing of glochidial release is under genetic control.

Due to high host specificity and the population's dependence on the fish for dispersal, the mussels are initially distributed over the habitat of the original host. If the original host spends 90% of its time in its preferred habitat, then about 90% of the mussel population should also occur there.

If the new host occurs, even infrequently, in the habitat of the mussels during their breeding period, these fish could serve as the host for mussels that possess the mutant phenotype (*i. e.* those able to parasitize the new host). Such mussels will begin slowly to accumulate (over many generations) in the new host's habitat, and the result will be at least partial habitat separation of the two phenotypes.

Because of the limited range of fertilization (see above), the tendency would be for adjacent mussels (*i. e.* within the same habitat) to interbreed. Thus, host specificity not only biases the habitat in which a mussel lives but also the phenotype of its mates. ***Mussels with a particular host specificity will mate more frequently with mussels sharing the same specificity (homogamy) because of their proximity.*** Linkage of mate and habitat "preferences" is the primary assumption of theories proposing reproductive isolation without geographical separation (Maynard Smith, 1966), but it is also a major bone of contention of the classic allopatric-geographic speciation paradigm (Mayr, 1947).

Temporal differences between the presence of the two fishes would contribute selection pressure towards the isolation of the two mussel phenotypes. Those mussels whose glochidial release coincides with peak host availability will have an obvious advantage over those that release their glochidia at other times; this would lead to synchronization of glochidial release with host presence. Kat (1984) has suggested that synchronization of mussel reproduction and fish activity is among the least specialized adaptations that unionoideans have evolved to increase the probability of glochidia encountering an appropriate host.

Fig. 1 shows an overview of the speciation model. At Stage A, the mussel population is distributed over the habitat range of the original host. New individuals are added to the population and tend to remain in the fish's preferred habitat. Between Stages A and B, a mutation appears that allows some members of the population to infect the new host. During Stage B, the mussels possessing the mutant phenotype accumulate in the habitat of the new host. Stage B can last many generations as the number of glochidia produced each generation that survive to reproductive age is very small.

Successful mating can occur in the new habitat after the mussels have accumulated to the point that their density is conducive to fertilization (Downing *et al.*, 1993); this marks the beginning of Stage C. Interbreeding occurs within but not between the habitats and there is a tendency for glochidia produced to remain in the habitat in which they were conceived. Thus, gene flow between the habitats is limited.

Over time, selection synchronizes the breeding of these two phenotypes with the habits of their respective host fish. This further decreases the amount of gene flow between the two incipient host races. Selection can accelerate this process by contributing to the spawning asynchrony of the two host races. Eventually, Stage D is reached when the two breeding types have become completely isolated due to their opposite host and habitat affinities.

The biological basis for the mutation in host specificity is immunological. It might also be reasonable, however, that the initial change in host specificity is due to a mutation that changes the time of glochidial release, with the habitat preferences of the host contributing selection towards separation. Conceptually, this would require a minor modification of the assumptions, but the same model remains applicable.

EXAMPLES

There are no unequivocal examples in the literature. Finding examples of populations in the early stages of this type of speciation process has proven especially difficult; a simultaneous examination of a mussel population's genetics and host preferences has yet to be undertaken. Provided for illustration are two unionoidean populations that could be candidates for just such a future study.

The first example involves *Anodonta woodiana* (Lea, 1834) in Japan. The "population" is composed of genetically and morphologically distinguishable sympatric morphs (A and B) (Tabe *et al.*, 1994). Further distinguishing these mussels is their breeding period. Morph A is tachytictic and releases its glochidia in late spring and early summer, while Morph B mussels are bradytictic with glochidial release occurring in the early spring (Fukuhara *et al.*, 1994). The reported fish hosts for *A. woodiana* are a goby and at least one cyprinid, and it has been shown that different hosts of this species are associated with different periods of mussel gravidity (Watters, 1994a).

Elliptio waccamawensis (Lea, 1863), a second example, is endemic to Lake Waccamaw and the Waccamaw River of the Atlantic Slope drainage of North Carolina (Johnson, 1970). Electrophoretic studies of members of the genus *Elliptio* indicate that this mussel is most closely related to *Elliptio cistelliformis* (Lea, 1863), which is also found in the lake as well as surrounding drainages (Davis *et al.*, 1981). Davis and co-workers (1981) reported

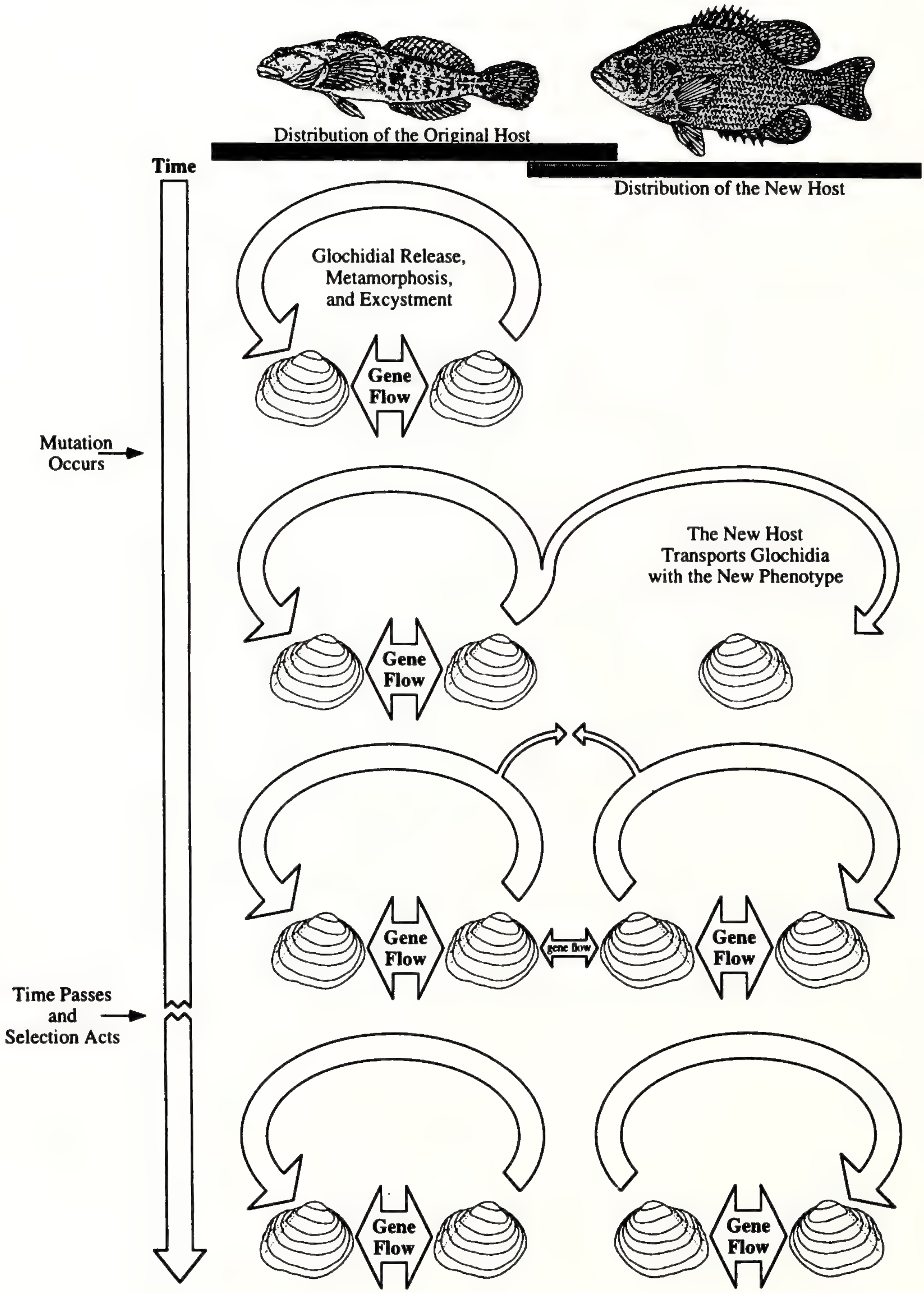
Distribution of the Original Host
Distribution of the New Host
Time
Glochidial Release, Metamorphosis, and Excystment
A
Gene Flow
Mutation Occurs
The New Host Transports Glochidia with the New Phenotype
B
Gene Flow
C
Gene Flow
gene flow
Gene Flow
Time Passes and Selection Acts
D
Gene Flow
Gene Flow

Fig. 1. Overview of the model of mussel speciation via host race formation. See text for discussion.

a distinct ecological difference between the two species, and I would predict that the fish host of *E. waccamawensis* is one of the endemic fishes of the Waccamaw drainage.

DISCUSSION AND CONCLUSIONS

Besides the two examples cited above, many other unionoidean taxa meet the criteria of this model for sympatric speciation via new host acquisition, including the limitation to one or a few host fish that share a common habitat preference. This appears contradictory to the widely held notion (*e. g.* Kat, 1984:199) that "... host specificity among unionaceans seems to be rather low." However, a critical examination of Hoggarth (1992) reveals that, of those associations confirmed by actual glochidial metamorphosis, greater than 73% of the 60 unionids reviewed are known to parasitize fish belonging to two or fewer genera. That is, for the parasite-host relationships thus far determined, the Unionoidea exhibit rather high fidelity to particular host genera. Within many of the host genera implicated, congeners share similar habitats and habits (K. Hartel, pers. comm.), and as it concerns the model, host specificity refers not so much to the number of species utilized but to the number of habitats frequented by those fishes.

The shortcoming of most models of ecological speciation is the difficulty of explaining how adapting to a new niche would lead to reproductive isolation. Under the assumption that the habitat preference of an organism could be changed by a mutation at a single locus, random interbreeding would tend to swamp the effects of the gradual accumulation of ecological separation between the two phenotypes (Mayr, 1947, 1970). The model presented here, however, is not based on the progressive acquisition of isolation. The fish hosts possess genetically hard-wired habitat preferences, and the mussels can capitalize on the niche fidelity fine-tuned during the evolution of the fish. Short effective fertilization distance completes the picture.

I do not argue that reproductive isolation via new host acquisition is a common mode of speciation in the Unionoidea. However, I would suggest that theories of speciation by geographical separation alone fail satisfactorily to explain the zoogeography and diversity of all mussel species in the Mississippi basin; the allopatric paradigm has yet to be corroborated by vicariance with the diversity of other families of aquatic organisms which would presumably reflect the same isolating events. Further, the habitat separation of genotypes achieved through the action of a shift in host fish could contribute to allopatric speciation following the erection of extrinsic barriers that subdivide populations (*e. g.* stream capture, etc.). Sympatric speciation via new host acquisition should be considered a viable alternative to allopatric speciation in the Unionoidea, and it is a mechanism in need of further theoretical and experimental testing.

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Freshwater mussels (Bivalvia: Unionidae) in the Verdigris, Neosho, and Spring River basins of Kansas and Missouri, with emphasis on species of concern

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Abstract: We examined freshwater mussel assemblages at 99 sites from 1993 to 1995 in the Arkansas River system of southeastern Kansas and southwestern Missouri. Emphasis was placed on assessing the distribution, relative abundance, and habitat use of five unionid candidates for future federal listing (species of concern): *Lampsilis rafinesqueana* Frierson, 1927, *Ptychobranchus occidentalis* (Conrad, 1836), *Cyprogenia aberti* (Conrad, 1850), *Quadrula cylindrica* (Say, 1817), and *Alasmidonta marginata* Say, 1818. We collected a total of 15,068 mussels of 35 species, including 1,301 *L. rafinesqueana*, 83 *P. occidentalis*, 29 *C. aberti*, seven *Q. cylindrica*, and one *A. marginata*. The three most abundant species collected from our study were *Amblema plicata* (Say, 1817), *Q. metanevra* (Rafinesque, 1820), and *Q. pustulosa* (Lea, 1831). However, species abundance rankings varied from stream to stream; for example, *L. rafinesqueana* was the most abundant species collected in the Spring River. Habitat use by candidate species varied considerably between streams, however, they were consistently found in shallow riffles and runs (mean depths 25.0-33.7 cm), with stable and moderately compacted substratum, predominantly gravel, with a minimum of silt.

Key Words: Unionidae, species of concern, freshwater mussels, Arkansas River system

Prompted by concern for diminishing freshwater mussel populations, the U. S. Fish and Wildlife Service (USFWS) listed six unionid species native to the Arkansas River system of southeastern Kansas and southwestern Missouri as candidates ("Category 2") for possible addition to the list of U. S. Endangered and Threatened Wildlife (15 November 1996, *Federal Register* 59(219):58982-59028). [Note: USFWS has discontinued the listing of "Category 2" candidate species, and has since labeled them as species of concern (05 December 1996, *Federal Register* 61:64481-64485)]. These species of concern are the Neosho mucket (*Lampsilis rafinesqueana* Frierson, 1927), Ouachita kidneyshell [*Ptychobranchus occidentalis* (Conrad, 1836)], western fanshell [*Cyprogenia aberti* (Conrad, 1850)], rabbitsfoot [*Quadrula cylindrica* (Say, 1817)], elktoe (*Alasmidonta marginata* Say, 1818), and purple lilliput [(*Toxolasma lividus* (Rafinesque, 1831)].

Lampsilis rafinesqueana is endemic to the Arkansas River system (Neosho, Spring, Elk, Illinois, and Verdigris River basins) in Kansas, Missouri, Oklahoma, and Arkansas (Gordon and Brown, 1980; Johnson, 1980; Oesch, 1984; Harris and Gordon, 1987; Mather, 1990; Stewart, 1992; Obermeyer *et al.*; 1995; Clarke and Obermeyer, 1996). Although populations of *L. rafinesqueana* persist within these states, its range has declined (Cope, 1979; Metcalf, 1980; Mather, 1990; Stewart, 1992; Clarke and Obermeyer, 1996; Obermeyer *et al.*, 1995, 1997). *Ptychobranchus occidentalis* is confined to the Arkansas, Black, Red, St. Francis, and White River systems in Arkansas, Kansas, Missouri, and Oklahoma (Valentine and Stansbery, 1971; Johnson, 1980). Although Buchanan (1980) and Oesch (1984) reported *P. occidentalis* in the Meramec River basin of Missouri (*i.e.* Upper Meramec River near the mouth of Blue Springs Creek), both (A. C. Buchanan, pers. comm.; R. D. Oesch, pers. comm.) have discounted this locality account due to suspected specimen mislabeling. *Cyprogenia aberti* is native to the Arkansas, Black, St. Francis, Ouachita, and White River systems in Arkansas, Kansas, Missouri, and Oklahoma (Johnson, 1980; Oesch, 1984; Harris and Gordon, 1987; Stewart, 1994). Its previously reported presence in the Meramec River basin of Missouri (*e.g.* Buchanan, 1980; Oesch, 1984) is now considered in error due to the same suspected mislabeling of specimens mentioned for *P. occidentalis*. *Cyprogenia aberti* is currently found in 14 streams in Arkansas, five in Missouri, and three

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in Kansas (Stewart, 1994); it is considered extirpated in Oklahoma (Mather, 1990). *Quadrula cylindrica* is native to the Ozarkian and Cumberland faunal regions (Johnson, 1980) of 13 states (Williams *et al.*, 1993), perhaps reaching its greatest abundance in the Black River system of Arkansas (D. H. Stansbery, pers. comm.). A subspecies, *Q. cylindrica strigillata* (Wright, 1898), which is considered by some as an ecomorph (*e.g.* Simpson, 1914; Gordon and Layzer, 1989; Clarke and Obermeyer, 1996) (but see Ortmann, 1920), occurs in the Clinch, Powell, and Holston Rivers of the Upper Tennessee River drainage (Ortmann, 1920; Bogan and Parmalee, 1983; Yeager and Neves, 1986). *Alasmidonta marginata* is widely distributed throughout eastern North America, being found in 22 states and one Canadian province (Clarke, 1981; Williams *et al.*, 1993). *Toxolasma lividus* is found in the Ohioan, Cumberlandian, and Ozarkian faunal regions (Johnson, 1980) of 12 states (Williams *et al.*, 1993).

The primary objectives of this study were to assess the distribution, abundance, and habitat use of five of the six species of concern mentioned above (*i.e.* all except *Toxolasma lividus*) in the Arkansas drainage system in eastern Kansas and southwestern Missouri (Neosho, Verdigris, and Spring River basins). However, we also noted the composition of the total mussel assemblage.

STUDY AREA

The Neosho and Verdigris River basins are situated within the tallgrass prairie ecoregion in southeastern Kansas. Cross and Collins (1995) termed the lotic waters of these two basins as Ozark-border streams, and characterized them as having the greatest habitat diversity for fishes in Kansas. The greatest richness of Kansas' unionid fauna also occurs within these basins – 37 species (Obermeyer *et al.*, 1997). Both basins are primarily agricultural, with native rangeland in many headwater reaches, whereas extensive cultivation occurs on and near the flood plains of tailwater reaches. Chert-gravel, derived of Permian and Pennsylvanian limestones (Wilson, 1984; Aber, 1992), is the dominant substratum of shallow riffle habitats. Principal streams of the Neosho and Verdigris River basins along with their respective drainage area (km^2) in Kansas follow: the Neosho (15,000) and Cottonwood (4,940) Rivers of the Neosho River basin, and the Verdigris (8,690), Fall (2,290), and Elk (1,820) Rivers in the Verdigris River basin (Fig. 1). Despite their size, these streams are subject to periodic flow interruptions during severe droughts (Deacon, 1961; Geiger *et al.*, 1995; Miller and Obermeyer, 1997). Recent flow disruptions have resulted from the construction and operation of several federal flood-control impoundments: Council Grove Lake and John Redmond Reservoir (Neosho River), Marion Lake (Cottonwood River), Fall River Lake (Fall River), Toronto Lake (Verdigris River), and Elk City Lake (Elk River) (Fig. 1).

Streams in the Spring River basin (Fig. 1), excluding the North Fork Spring River, which is a prairie stream (Davis and Schumacher, 1992), originate from the northwestern flank of the Ozark Uplift. The basin's flow is generally westward until reaching Kansas, where it turns southward into Oklahoma (Davis and Schumacher, 1992), eventually joining the Neosho River. The Spring River basin drains approximately 5,415 km^2 of southwestern Missouri, and an additional 1,370 km^2 in southeastern Kansas (Davis and Schumacher, 1992). Streams examined in the Spring River basin included the Spring and North Fork Spring Rivers, and Shoal and Center Creeks. These streams differ from Ozark-border streams by having lower turbidity, richer aquatic faunas (Cross and Collins, 1995), and flows sustained by headwater springs during droughts. Land use in several of these streams also differs from that in the Neosho and Verdigris basins in that a sizable proportion of the drainage area is forested (*e. g.* 45% for Shoal Creek; Davis and Schumacher, 1992). In addition, extensive lead and zinc mining has occurred in the Spring River basin, which has especially affected the lower Spring River and Shoal Creek in Kansas and Center Creek in Missouri (Kansas Department of Health and Environment, 1980; Davis and Schumacher, 1992). Furthermore, these streams lack the large flood-control impoundments that have altered streams in the Neosho and Verdigris basins (Obermeyer *et al.*, 1997). The mussel assemblage of the Spring River basin differs from that of the Neosho and Verdigris River basins in having the following species: *Alasmidonta marginata, A. viridis* (Rafinesque, 1820), *Fusconaia ozarkensis* (Call, 1887), *Toxolasma lividus* (Rafinesque, 1831), and *Venustaconcha ellipsiformis* (Conrad, 1836) (Gordon and Brown, 1980; Cope, 1985; Obermeyer *et al.*, 1995). Also, four species present in the latter basins are absent from the Spring River basin: *Ellipsaria lineolata* (Rafinesque, 1820), *Truncilla donaciformis* (Lea, 1827), *T. truncata* Rafinesque, 1820, and *Megalonaias nervosa* (Rafinesque, 1820) (Cope, 1985; Obermeyer *et al.*, 1995).

METHODS

SAMPLING

Sampling sites were confined to streams in the Arkansas River Basin with known accounts of one or more of the targeted species. An attempt was made to space sample sites evenly within each stream; however, unsuitable habitat in some stream stretches (*e. g.* unstable banks, bedrock substratum) and/or difficulty in securing legal access sometimes made this impossible. We also tried to

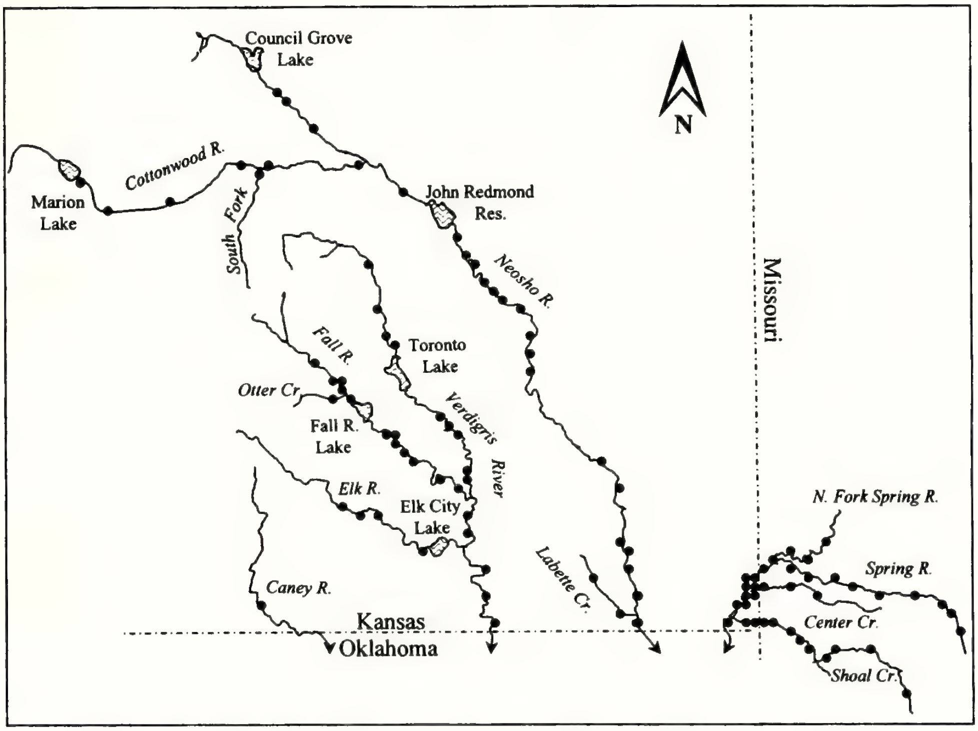

Fig. 1. Sampling sites in southeastern Kansas and southwestern Missouri.

sample sites examined by previous surveyors; unfortunately, many of these sites lacked exact locality data.

To locate living mussels in shallow water (15 cm to < 1 m) with adequate visibility, we used a snorkel and face mask, whereas at depths exceeding 1 m, SCUBA was used. Mussels were located both by tactile cues (groping) and by visual cues during snorkeling and SCUBA searches. We also visually searched for mussels in shallow habitats as well as recently exposed substratum. Sampling was concentrated in riffles and runs; however, runs and pools were also examined to assess usage of these habitats. All searches were timed to quantify sampling effort, which ranged from 40 min to 9 h, depending on quantity and quality of habitat. Weather conditions and water levels also influenced sampling effort.

We also quantitatively examined 14 sites in Kansas (Neosho = 9, Spring = 2, Fall = 3) using a 1-m^2 quadrat; a total of 505 quadrats was sampled at these sites. Quadrats were placed along measured coordinates chosen randomly, with the substratum excavated by hand to an approximate depth of 10-15 cm.

To seek evidence of young recruits, we sampled substratum from habitats cited as being most often utilized by juveniles (Isely, 1911; Clarke, 1986; Neves and Widlak, 1987); substratum was dredged with a shovel and transferred to a 1-m^2 sieve (6-mm mesh) supported by a floating 15-cm PVC-pipe frame. Dredging ceased when the weight of the substratum caused the frame to sink. The substratum was then sieved in an attempt to locate small mussels. The number of sieve samples examined at each site varied from 0 to 21.

Except for a few specimens collected for reference, living unionids were identified in the field, measured with either a dial caliper or an aluminum plate shell-sizer with openings of 2, 3, 4, 6, 8, 10, and 12 cm (Obermeyer, 1996a), and returned to their original location. Reference shells from sites sampled in 1994 are deposited in the Ohio State University Museum of Zoology in Columbus, Ohio, and vouchers from 1995 sites will be housed at the Kansas Biological Survey, University of Kansas, Lawrence. Nomenclature of unionids follows Turgeon *et al.* (1988); however, subgenera *Utterbackia* Baker, 1927, and *Pyganodon* Crosse and Fischer, 1893, are elevated to generic status following Hoeh (1990), and *Fusconaia ozarkensis* and *F. flava* (Rafinesque, 1820) collected from the Spring River and Shoal Creek are listed as *Fusconaia* spp. due to identification uncertainties. Nomenclature of fishes follows Robins *et al.* (1991).

HABITAT CHARACTERIZATION

At specific locales where living individuals of candidate species were found, we made visual estimates of three substratum variables: substratum compaction, percent composition of substratum types, and silt deposition on the substratum. Substratum compaction was coded as 0, 1, or 2, with 0 being loose, 1 moderately compacted, and 2 very compacted. Substrata were divided into five approximate size classes: mud (< 0.8 mm), sand (0.8 - 4 mm), gravel (4 - 50 mm), cobble (50 - 290 mm), and boulder (> 290 mm) (modified from Platts *et al.*, 1983). We coded the degree of silt deposition from 0 to 3, where 0 characterized a clean substratum, 1 had a detectable silt layer, 2 was moderately covered with silt, and 3 was heavily silt-laden. Current speed and water depth were measured for each candidate specimen with a pygmy Gurley current meter no. 625 at 60% depth and at the substratum-water interface (100% depth).

RESULTS

From a combined effort of 505 1-m^2 quadrats and approximately 200 h of qualitative sampling from 99 sites in the Arkansas River system (Neosho River basin = 30 sites; Verdigris River basin = 32 sites; Spring River basin = 37 sites), we collected 15,068 living mussels representing 35 species (Table 1). *Corbicula fluminea* (Müller, 1774), a recent bivalve invader (Corbiculidae), was also found in all streams. Over 9% of our collections consisted of species of concern, with 1,301 *Lampsilis rafinesqueana*, 83 *Ptychobranchus occidentalis*, 29 *Cyprogenia aberti*, seven *Quadrula cylindrica*, three *Toxolasma lividus*, and one *Alasmidonta marginata* collected. The most abundant species encountered during the survey was *Amblema plicata* (Say, 1817), comprising 18.9% of the total sample, followed by *Quadrula metanevra* (Rafinesque, 1820) and *Q. pustulosa* (Lea, 1831), representing 18.2% and 11.8%, respectively (Table 1). However, species rank varied among basins and streams; *Q. metanevra, A. plicata*, and *Q. pustulosa* were the three most common species in the Neosho River Basin, *A. plicata, Q. pustulosa*, and *Q. metanevra* the most numerous in the Verdigris River basin, and *L. rafinesqueana, Fusconaia* spp., and *Elliptio dilatata* (Rafinesque, 1820) the most common in the Spring River basin (Table 1).

Although *Lampsilis rafinesqueana* was the fourth most abundant species encountered in this study (8.6% of total sample), most of these individuals (1192 = 91.6%) were collected from the Spring River, representing 40.2% of the Spring River total (Table 1). This species was found alive at 13 of 20 Spring River sites, from just downstream of state Highway 97 bridge near Stott City, Lawrence County, Missouri, to the confluence of Turkey Creek, Kansas (Fig. 2). It was the most abundant species encountered at 11 of these sites. In Shoal Creek, 26 *L. rafinesqueana* were collected at five of 11 sites (Table 1; Fig. 2), but only in the Missouri portion of this stream. Two of three North Fork Spring River sites yielded 12 *L. rafinesqueana* specimens (Table 1; Fig. 2). This species was not collected alive in Center Creek, but one recently dead specimen was recovered. In the Neosho River, 32 *L. rafinesqueana* were collected at six of 21 sites, representing 0.6% of this river's collection (Table 1); these were all found downstream from John Redmond Reservoir (Fig. 2). In the Verdigris River, a total of five *L. rafinesqueana* was found at four of 14 sites (0.2% of the total Verdigris River sample; Table 1); all four of these sites were located downstream from Toronto Lake and upstream from the confluence of the Elk River (Fig. 2). Thirty-four *L. rafinesqueana* were collected at five of 12 Fall River sites between Fall River Lake and the confluence of the Verdigris River (Table 1; Fig. 2), representing 1.7% of the total sample from this stream. Although weathered shells of this species were observed at sites in the Cottonwood, Caney, and Elk Rivers, living or recently dead specimens were not found (Table 1).

Young *Lampsilis rafinesqueana*, either living or freshly dead, were found at few sites. Based on external estimations of annuli, most Verdigris and Neosho basin specimens were over 20 years old; only three specimens collected in these two basins were estimated to be of young age (6-10 years). Spring River basin specimens were comprised mostly of two or three cohorts between eight and 20 years of age; the youngest *L. rafinesqueana* specimens collected alive or as recently dead specimens were four years old, the smallest being a recently dead specimen from Shoal Creek that measured 49 x 32 x 16 mm (length, height, and width, respectively). In the Neosho and Verdigris River basins, mean length for caliper-measured *L. rafinesqueana* was 131.2 mm (SD = 12.96), with specimens ranging 94 - 163 mm. Spring River *L. rafinesqueana*, which were measured with the aluminum shell-sizer, averaged 110.8 mm (SD = 11.10), whereas caliper-measured Shoal Creek specimens were considerably smaller (mean = 72.5 mm, SD = 8.73).

Lampsilis rafinesqueana was collected most often in shallow riffles and runs having predominantly gravel substratum (Table 2); however, there was a substantial difference in habitat use by *L. rafinesqueana* in the Spring River and Shoal Creek compared to that in the Neosho, Fall, and Verdigris Rivers (Table 2). For instance, mean current speed at locales utilized by *L. rafinesqueana* was much higher in the Spring River basin than in prairie streams (Table 2). The mean coded value for silt deposition at *L. rafinesqueana* sites in the Spring River was 0.2 (SD = 0.40) compared to 1.4 (SD = 0.50) in the Neosho, Verdigris, and Fall Rivers (Table 2). These data are likely variant due to the uniqueness of the Spring River compared to other Kansas streams (Cross and Collins, 1995), and because of greater *L. rafinesqueana* densities in the Spring

Table 1. Unionid mussels collected in 1993-1995 from the Neosho, Spring, and Verdigris River basins in southeastern Kansas and southwestern Missouri, and the contribution of each stream. [d, dead (recent); Lr, literature record; wd, weathered dead].

		Neosho Basin				Spring Basin				Verdigris Basin				
Species	Streams & No. sites: Totals	Neosho R. 21	Cottonwd. R. 6	S. Fork Cwd. R. 1	Labette Cr. 2	Spring R. 20	N. Fork Spring R. 3	Center Cr. 3	Shoal Cr. 11	Verdigris R. 14	Fall R. 12	Elk R. 4	Otter Cr. 1	Caney R. 1
Alasmidonta marginata Say, 1818	**1**	–	–	–	–	1	–	–	wd	–	–	–	–	–
A. viridis (Rafinesque, 1820)	**1**	–	–	–	–	d	–	–	1	–	–	–	–	–
Amblema plicata (Say, 1817)	**2844**	1274	d	wd	91	94	132	wd	47	688	461	57	d	d
Cyprogenia aberti (Conrad, 1850)	**29**	Lr	–	–	–	13	–	–	–	11	5	wd	–	Lr
Ellipsaria lineolata (Rafinesque, 1820)	**87**	80	–	–	–	Lr	–	–	–	7	Lr	–	–	–
Elliptio dilatata (Rafinesque, 1820)	**539**	179	d	wd	–	280	23	1	56	Lr	–	–	–	–
Fusconaia flava (Rafinesque, 1820)	**860**	334	1	wd	12		26			219	217	51	wd	d
Fusconaia spp.*	**440**	–	–	–	–	372	–	d	68	–	–	–	–	–
Lampsilis cardium (Rafinesque, 1820)	**499**	103	d	d	–	54	27	13	54	106	128	14	d	d
L. rafinesqueana Frierson, 1927	**1301**	32	wd	Lr	–	1192	12	d	26	5	34	wd	–	wd
L. siliquoidea (Barnes, 1823)	**23**	Lr	–	–	–	8	12	2	Lr	wd	d	wd	wd	1
L. teres (Rafinesque, 1820)	**71**	16	d	wd	5	1	7	–	Lr	16	20	6	wd	wd
Lasmigona complanata (Barnes, 1823)	**151**	14	d	d	16	3	3	–	–	78	29	8	d	d
L. costata (Rafinesque, 1820)	**31**	3	wd	wd	–	28	wd	wd	–	wd	wd	wd	Lr	Lr
Leptodea fragilis (Rafinesque, 1820)	**172**	113	6	d	3	Lr	–	–	–	24	23	3	d	d
Ligumia recta (Lamarck, 1819)	**wd**	wd	wd	wd	–	wd	–	–	wd	wd	wd	wd	wd	Lr
L. subrostrata (Say, 1831)	**18**	d	d	wd	2	2	1	–	9	wd	4	d	d	d
Megalonaias nervosa (Rafinesque, 1820)	**209**	198	wd	–	–	Lr	–	–	–	8	3	–	–	–
Obliquaria reflexa Rafinesque, 1820	**490**	292	d	–	9	wd	–	–	–	133	47	9	–	–
Pleurobema coccineum (Conrad, 1834)	**421**	30	wd	–	–	335	–	wd	7	40	9	wd	wd	–
Potamilus ohiensis (Rafinesque, 1820)	**5**	3	d	–	–	–	–	–	–	2	–	d	–	d
P. purpuratus (Lamarck, 1819)	**183**	103	d	1	6	1	–	–	–	29	23	20	d	d
Ptychobranchus occidentalis (Conrad, 1836)	**83**	wd	wd	wd	–	45	2	–	6	11	19	wd	wd	wd
Pyganodon grandis (Say, 1829)	**14**	2	d	–	1	2	2	d	d	d	7	d	wd	d
Quadrula cylindrica (Say, 1817)	**7**	2	wd	–	–	5	–	–	wd	wd	wd	–	–	–
Q. metanevra (Rafinesque, 1820)	**2748**	1786	wd	–	–	15	–	–	1	658	288	d	–	–
Q. nodulata (Rafinesque, 1820)	**42**	12	Lr	wd	–	Lr	–	–	–	24	6	–	–	–
Q. pustulosa (I. Lea, 1831)	**1779**	537	5	d	30	243	13	wd	d	388	485	78	d	d
Q. quadrula (Rafinesque, 1820)	**683**	274	18	1	53	60	34	–	wd	130	84	29	d	d
Strophitus undulatus (Say, 1817)	**162**	7	d	d	–	14	68	1	2	35	24	11	wd	d
Toxolasma lividus (Rafinesque, 1831)	**3**	–	–	–	–	–	–	–	3	–	–	–	–	–
T. parvus (Barnes, 1823)	**3**	Lr	–	–	–	–	–	–	Lr	1	1	–	–	1
Tritogonia verrucosa (Rafinesque, 1820)	**893**	354	29	d	16	76	33	–	–	160	189	35	1	d
Truncilla donaciformis (I. Lea, 1828)	**62**	25	d	d	d	–	–	–	–	8	29	d	–	Lr
T. truncata Rafinesque, 1820	**6**	d	wd	–	–	Lr	–	–	–	6	wd	wd	–	d
Uniomerus tetralasmus (Say, 1831)	**d**	d	–	–	d	–	–	–	Lr	Lr	–	wd	–	Lr
Utterbackia imbecillis (Say, 1829)	**d**	Lr	–	–	–	–	–	–	d	Lr	d	–	–	d
Venustachoncha ellipsiformis (Conrad, 1836)	**208**	–	–	–	–	120	61	7	20	–	–	–	–	–
Totals	**15068**	5773	59	2	244	2964	456	24	300	2787	2135	321	1	2

**Fusconaia* spp. represents the *Fusconaia flava* (Rafinesque, 1820) and *F. ozarkensis* (Call, 1887) complex.

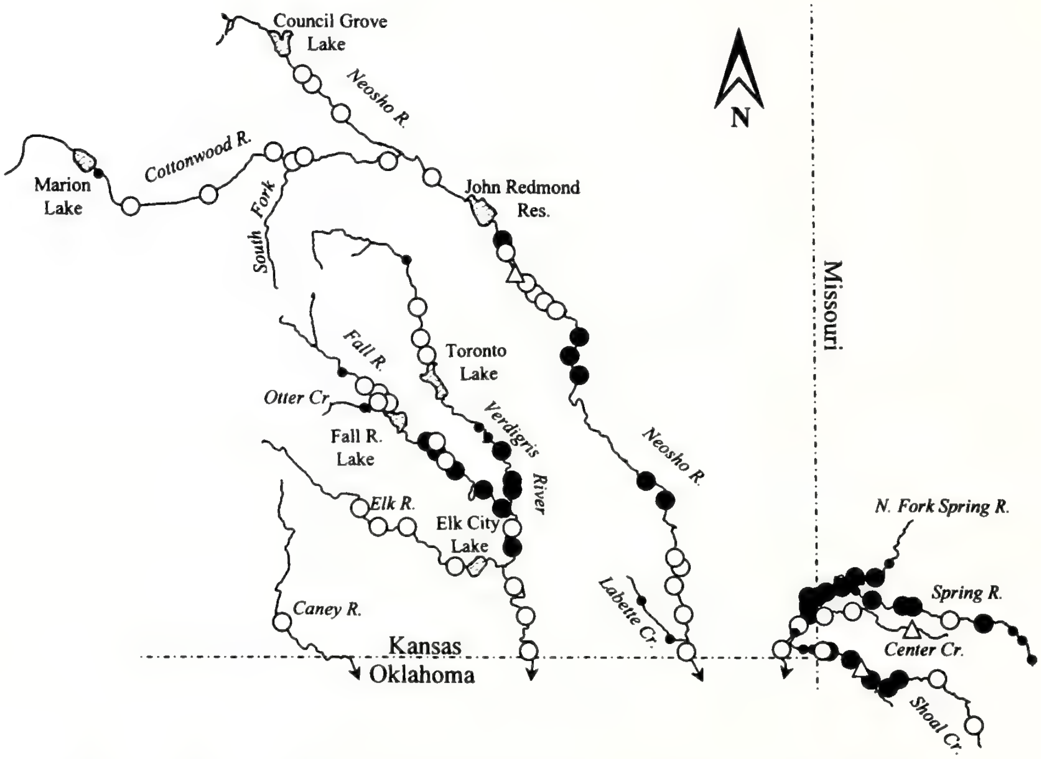

Fig. 2. Range map of *Lampsilis rafinesqueana* in the Neosho, Spring, and Verdigris River basins in southeastern Kansas and southwestern Missouri. Solid circles indicate sites where living specimens were found, triangles represent recently dead specimens, open circles are sites yielding only weathered and/or relic valves, and small solid dots represent sites in which we did not find evidence of the species.

River. For example, 67 *L. rafinesqueana* were collected in one 1-m^2 quadrat (located at a depth of 28 cm with current speeds of 90 and 68 cm/s at 60 and 100% depth, respectively, in clean, moderately loose substratum consisting of 10% sand, 80% gravel, and 10% cobble); whereas the species was found only sporadically in other Kansas streams.

Ptychobranchus occidentalis ranked nineteenth in relative abundance from collections in the three basins (Table 1). This species was not collected alive in the Neosho River, despite abundant weathered valves at several sites. In the Verdigris River, 11 *P. occidentalis* were found at four sites (Table 1; Fig. 3), representing 0.4% of this stream's total sample. Nineteen specimens were collected at six Fall River sites (Table 1; Fig. 3), comprising 0.9% of unionids collected in this stream. In the Spring River, we collected 45 *P. occidentalis* at ten sites, representing 1.5% of the collection (Table 1; Fig. 3). We also found two specimens at one site in the North Fork of the Spring River, and six individuals at a Shoal Creek site in Missouri (Table 1; Fig. 3). In the Cottonwood, Elk, Caney, and South Fork Rivers, only weathered shells of this species were noted (Table 1). Most *P. occidentalis* specimens were over seven years old. The youngest noted was a recently dead three-year-old specimen (41 x 20 x 9 mm) collected from a Fall River site. Mean shell length for *P. occidentalis* in the Verdigris basin was 90.2 mm (SD = 20.74), whereas Spring River basin specimens were slightly larger (mean = 97.4 mm, SD = 16.17). Like *Lampsilis rafinesqueana, P. occidentalis* exhibited differences in habitat use among streams (Table 2); however, it was found predominantly in riffles.

Cyprogenia aberti was collected alive in only three streams, representing 0.2% of the total sample. In the Verdigris River, we collected 11 *C. aberti* at five sites, in the Fall River five specimens were found at four sites, and in the Spring River we collected 13 specimens at six sites (Table 1; Fig. 4). We also found one relic *C. aberti* valve from the Elk River (Table 1; Fig. 4), which represents a new stream record. Although *C. aberti* was documented in the Neosho River by both Call (1885a) and Scammon (1906), we were unable to find evidence of this species, either recent or weathered valves, in this stream. Only four of the *C. aberti* we collected were less than five years old, all measuring less than 45 mm in length; the smallest measured 34 x 26 x 16 mm. A Verdigris River site also yielded a young freshly dead specimen in exposed gravel, which measured 44 x 35 x 15 mm; we estimated this specimen to be three years old. Shell length of living specimens from the Spring, Fall, and Verdigris Rivers ranged 34 - 81 mm

Table 2. Observed habitat use [mean (SD)] by candidate mussels from Arkansas Basin streams in Kansas and Missouri.

Stream	N	Depth (cm)	Current speed (cm/s) at:		Substratum character (%):					Compaction	Siltation
			100% depth	60% depth	Mud	Sand	Gravel	Cobble	Boulder		
Lampsilis rafinesqueana											
Fall R.	34	34.1 (20.9)	12.4 (10.7)	13.2 (8.3)	0.7 (1.8)	11.7 (12.3)	48.4 (22.5)	37.6 (24.3)	1.5 (4.2)	1.2 (0.6)	1.3 (0.5)
Verdigris R.	5	26.2 (18.9)	3.2 (4.6)	5.2 (7.3)	11.0 (16.3)	11.0 (5.7)	52.0 (18.2)	27.0 (17.2)	–	1.0 (0.0)	1.6 (0.5)
Neosho R.	32	39.6 (22.2)	16.0 (13.8)	27.0 (25.4)	3.3 (6.6)	14.9 (13.7)	41.3 (20.0)	35.9 (24.6)	4.4 (14.4)	1.1 (0.4)	1.4 (0.5)
Spring R.	258	33.0 (11.7)	43.5 (19.3)	72.4 (27.1)	1.0 (3.3)	16.4 (16.9)	74.3 (16.6)	–	–	1.0 (0.0)	0.2 (0.4)
Shoal Cr.	20	59.4 (15.4)	20.4 (11.5)	42.2 (28.4)	0.3 (1.1)	17.1 (7.1)	74.5 (14.8)	8.3 (16.9)	–	0.9 (0.4)	0.1 (0.2)
Ptychobranchus occidentalis											
Fall R.	17	17.5 (12.8)	12.2 (11.9)	14.1 (10.6)	1.8 (3.3)	15.3 (12.2)	62.0 (19.5)	13.9 (12.1)	6.9 (20.7)	0.9 (0.2)	1.2 (0.6)
Verdigris R.	9	19.0 (8.1)	13.2 (10.3)	18.6 (14.4)	2.6 (2.7)	15.3 (4.5)	73.2 (8.9)	8.9 (7.8)	–	1.0 (0.0)	1.3 (0.5)
Spring R.	12	41.0 (17.7)	26.8 (19.8)	44.4 (27.9)	1.0 (1.8)	24.6 (25.2)	69.0 (24.1)	5.4 (6.6)	–	0.9 (0.3)	0.3 (0.5)
Shoal Cr.	4	73.5 (4.0)	34.9 (7.1)	97.1 (6.4)	0.0 (0.0)	11.8 (2.4)	82.0 (3.6)	7.5 (5.0)	-	1.3 (0.3)	0.0 (0.0)
Cyprogenia aberti											
Fall R.	5	29.6 (17.6)	8.4 (7.9)	16.8 (12.1)	0.2 (0.5)	14.2 (15.2)	18.4 (25.3)	45.2 (29.5)	22.0 (31.9)	1.0 (0.0)	1.2 (0.5)
Verdigris R.	9	26.5 (26.)	17.1 (18.1)	20.9 (19.6)	4.1 (6.0)	12.6 (8.6)	7.3 (6.1)	75.1 (15.7)	–	0.8 (0.4)	1.5 (0.5)
Spring R.	3	37.3 (10.7)	27.2 (17.0)	65 (35.8)	–	30.0 (35.0)	1.7 (2.9)	68.3 (33.3)	–	0.7 (0.6)	0.3 (0.6)
Quadrula cylindrica											
Neosho R.	2	12.5 (13.4)	27.5 (16.3)	38.0 (31.1)	0.5 (0.7)	7.0 (4.2)	60.0 (8.5)	32.5 (3.5)	–	1.0 (0.0)	1.0 (0.0)
Spring R.	5	44.2 (16.6)	23.8 (9.1)	56.2 (31.9)	–	20.0 (12.7)	80.0 (12.7)	–	–	0.9 (0.2)	0.2 (0.4)

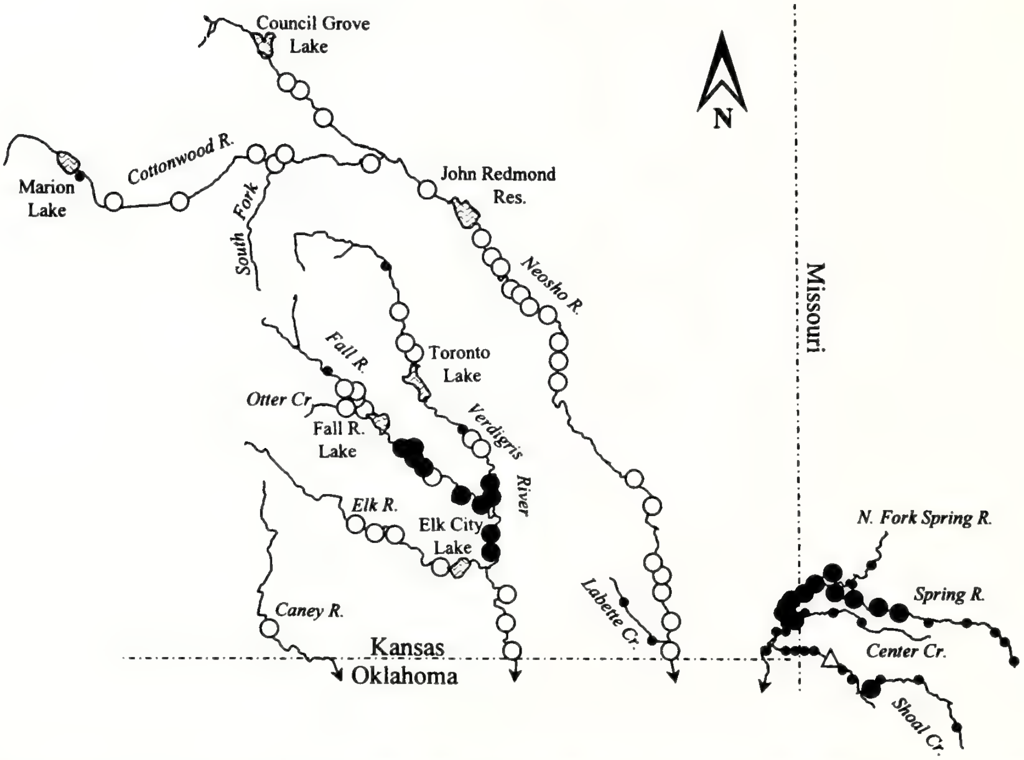

Fig. 3. Range map of *Ptychobranchus occidentalis* in the Neosho, Spring, and Verdigris River basins in southeastern Kansas and southwestern Missouri. Symbols as in Fig. 2.

(mean = 61.0 mm, SD = 13.40). *C. aberti* specimens collected in this study were generally confined to shallow riffles and runs in predominantly clean, moderately compacted gravel-sand substrata (Table 2).

Living representatives of *Quadrula cylindrica* were found in the Neosho and Spring Rivers (Table 1; Fig. 5), predominantly in shallow habitats with clean, moderately compacted gravel-sand substrata (Table 2). In the Neosho, we collected two living specimens from two sites (Table 1; Fig. 5), as well as two recently dead articulated specimens with desiccated softparts at one of these sites. Although freshly dead specimens of this species have been found in recent years in this stream (C. H. Cope, pers. comm.), these individuals are the first *Q. cylindrica* specimens reported alive from the Neosho River since 1912 (Isely, 1924). Relic *Q. cylindrica* valves were also found at nine additional Neosho River sites (Fig. 5). In the Spring River, we collected a total of five specimens at one Kansas and three Missouri sites (Table 1; Fig. 5). Relics were collected from one Shoal Creek site in Missouri, which represents the first evidence of this species in Shoal Creek. New stream records for *Q. cylindrica* were also made for the Fall and Cottonwood Rivers, with relic valves collected at two sites in each of these streams. Although Isely (1924) reported living *Q. cylindrica* in the Verdigris River in 1912, we found only relic valves of the species at eight of 14 sites (Fig. 5).

We estimated that three *Quadrula cylindrica* specimens from the Neosho River (two recently dead and one alive) were in their sixth year of growth (78, 86, and 87 mm long); an additional living specimen was estimated to be in excess of ten years old (113 mm long). A rather large specimen (recently dead valve) of this species, which measured 127 mm in length, was also recovered at one of these sites. In the Spring River, *Q. cylindrica* specimens ranged 74 - 109 mm in length (mean = 93.0 mm; SD = 12.71).

Only one *Alasmidonta marginata*, measuring 73 x 38 x 30 mm, was collected during the study, at a Kansas Spring River site (Table 1). It was found in riffle habitat with current speeds of 72 and 33 cm/s (60 and 100% depth, respectively) at 54 cm depth in predominantly cobble substratum (6% sand, 15% gravel, and 79% cobble). Weathered shells of this species were recovered at two additional Spring River sites in Kansas; one weathered valve was found at a Shoal Creek site in Missouri, the first account of this species in that stream.

Although *Toxolasma lividus* was not initially targeted in this study, we found three living individuals at one site in Shoal Creek in Missouri, as well as dead shells at three additional Shoal Creek sites (Table 1). The three living speci-

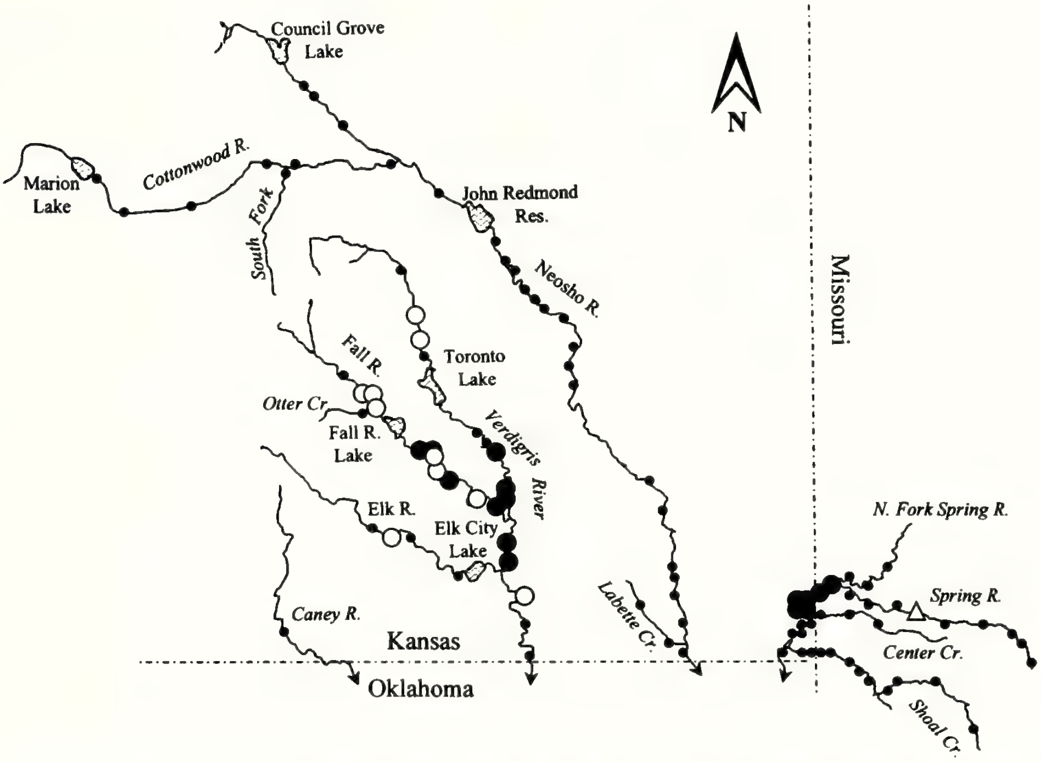

Fig. 4. Range map of *Cyprogenia aberti* in the Neosho, Spring, and Verdigris River basins in southeastern Kansas and southwestern Missouri. Symbols as in Fig. 2.

mens were found in a shallow backwater pool, which consisted of silty sediments with no detectable current.

DISCUSSION

Habitat descriptions for freshwater mussels have often been generalized (Gordon and Layzer, 1989). For example, *Quadrula cylindrica* occurring in medium to large streams is cited as preferring sand-gravel substrata in 6-10 feet of water (Parmalee, 1967; Cummings and Mayer, 1992) with a detectable current (Parmalee, 1967). However, in smaller streams the species is considered a riffle species, being most often found near shore in cobble substratum with a slack current (Stansbery, 1974) or, as Gordon and Layzer (1989) reported, in close proximity to the swiftest flows. Anecdotal descriptions of habitat use by *Ptychobranchus occidentalis* are gravel substratum in riffles with depths between 2.5 and 75 cm in slow to moderate current (Buchanan, 1980; Oesch, 1984); Gordon and Layzer (1989) stated that two congeners, *P. fasciolaris* (Rafinesque, 1820) and *P. subtentum* (Say, 1825), seem to prefer shallow riffles in moderate to swift currents. Habitat use by *Cyprogenia aberti* is described as shallow water (7-45 cm), with mud, sand, and gravel substrata (Murray and Leonard, 1962; Buchanan, 1980; Oesch, 1984). *Alasmidonta marginata* is reported to prefer riffles in cobble-gravel and gravel-sand substrata in medium to large rivers (Clarke and Berg, 1959; Clarke, 1981; Cummings and Mayer, 1992), with a preference for moderate to swift currents (Clarke and Berg, 1959; Gordon and Layzer, 1989). Oesch (1984) described the habitat use of *Lampsilis rafinesqueana* in Missouri as shallow water with a moderate current in fine to medium gravel.

More detailed habitat descriptions for unionids are difficult because of broad microhabitat tolerances (Strayer, 1981; Kat, 1982; Gordon and Layzer, 1989; Holland-Bartels, 1990; Strayer and Ralley, 1993; Strayer *et al.*, 1994), and because of site-specific preferences (Strayer, 1981) due to macro-scale variation (*e. g.* hydrologic variability) among sites (Strayer and Ralley, 1993). Habitat use by mussels collected in the present study was also variable when compared among different streams (Table 2), which made it difficult to extrapolate habitat suitability indices from one stream to another. Habitat use on a broader scale, however, was more predictable; that is, mussels were most often found in shallow riffles and runs at depths less than one meter, with stable and moderately compacted substratum, predominantly gravel, with a minimum of silt. Examination of deeper, more silt-laden habitats (*i. e.* pools) revealed a decrease in species richness and abundance of

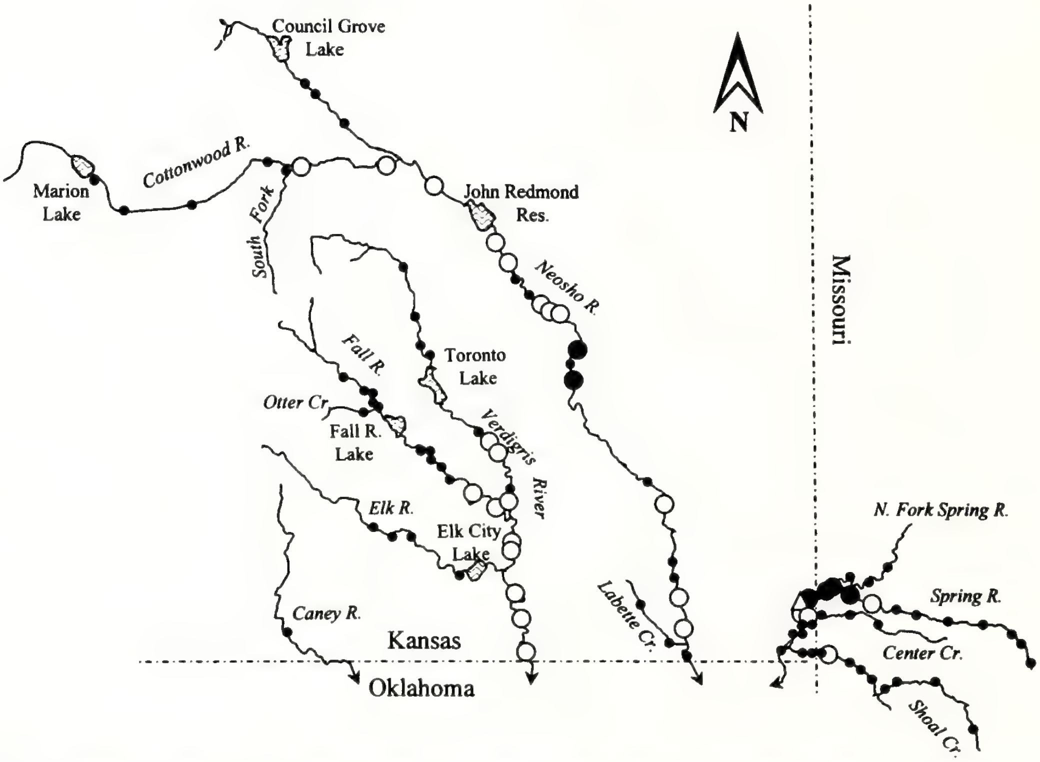

Fig. 5. Range map of *Quadrula cylindrica* in the Neosho, Spring, and Verdigris River basins in southeastern Kansas and southwestern Missouri. Symbols as in Fig. 2.

unionids, including the absence of candidate species. Sites that were unstable (*i. e.* loose, shifting substrata) were especially low in unionid numbers.

Of the five species targeted in this study, habitat use by *Lampsilis rafinesqueana* was especially intriguing. In the Neosho, Verdigris, Spring, and North Fork Spring Rivers, *L. rafinesqueana* was found most often in riffle habitat, usually in a swift current. However, in Shoal Creek, the species was often found in habitats near shore or out of the strongest current. Mather (1990) and C. C. Vaughn (pers. comm.) found a similar trend in another Ozarkian stream, the Illinois River in Oklahoma, and described the habitat most often associated with *L. rafinesqueana* in this stream as backwater areas. The rarity of *L. rafinesqueana* in mid-channel flows in Shoal Creek might be due to greater disruptions of substratum, especially during spates, than in other streams. Despite *L. rafinesqueana's* apparent inability to colonize extremely unstable habitats, this species seemed more adapted to unstable habitats than most other unionids. We observed that individuals of *L. rafinesqueana* in the Spring River and Shoal Creek often had their foot well extended into the substratum, especially in loose gravel. Kat (1982) similarly noted that *Elliptio complanata* (Lightfoot, 1786) used its foot to maintain a viable position in unstable habitats (*i. e.* soupy mud). Foot extension of *L. rafinesqueana* was seldom observed, however, in prairie streams of the Neosho and Verdigris basins and in the North Fork Spring River. Foot anchoring by *L. rafinesqueana* in the Spring River basin was probably due to swifter average current speeds in the Spring River basin versus the Neosho and Verdigris River basins (Table 2), and because substrata in the Spring River and Shoal Creek were less compacted than those in the prairie streams mentioned.

Lampsilis rafinesqueana has apparently become extirpated from six Kansas streams: the Elk, Caney, Cottonwood, and South Fork of the Cottonwood Rivers, and Shoal and Middle Creeks (Cope, 1979, 1985; Metcalf, 1980; Obermeyer, 1996b; Obermeyer *et al.*, 1997), and currently remains in the Verdigris, Fall, Neosho, and Spring Rivers (Cope, 1979, 1983, 1985; Miller, 1993; Obermeyer *et al.*, 1995; 1997) (Fig. 2). In addition to its range decline in Kansas, *L. rafinesqueana* seems to have become less abundant (Obermeyer *et al.*, 1997). It is presently listed in Kansas as endangered. In Missouri, *L. rafinesqueana* is confined to the Spring and Elk River basins (Gordon and Brown, 1980; Johnson, 1980; Oesch, 1984; Clarke and Obermeyer, 1996), and is state-listed as rare (Anonymous, 1994). Despite the decline of *L. rafinesqueana* in Kansas, the relative abundance of this species could have increased in the Spring River since Branson's (1967) survey. For example, Branson collected only 15 "muckets" at one of our Spring River sites in Kansas, whereas we found 112 living *L. rafinesqueana* [Note: His identifications of *Actinonaias ligamentina* (Barnes, 1823) were likely *L. rafinesqueana*]. Presently, the species is probably more abundant in the Spring River from Carthage, Missouri, to near the confluence of Center Creek, Kansas, than anywhere else throughout its range. Although *L. rafinesqueana* appears to remain within most of its historic range in Missouri, Branson (1967) recovered the species at sites farther upstream in both the Spring River and Shoal Creek, perhaps indicating a slight decrease in range. In the Kansas portion of the Spring River, *L. rafinesqueana*, as well as most other riverine mussel species, is apparently extirpated downstream from Turkey Creek (Fig. 2). Although *L. rafinesqueana* was previously reported as absent in the Spring River downstream from the confluence of Center Creek (Cope, 1985; Stewart, 1992; Obermeyer *et al.*, 1995), we collected this species at two riffle sites immediately downstream from Center Creek, which may indicate improving stream conditions.

Lampsilis rafinesqueana is a bradytictic breeder (Barnhart and Roberts, 1997), and females, like other lampsilines, attract potential hosts with a mantle lure (Johnson, 1980; Oesch, 1984; Barnhart and Roberts, 1997), with July and August being the period of most frequent mantle display (B. K. Obermeyer, pers. obs.). Two potential hosts have been identified for *L. rafinesqueana*: smallmouth bass (*Micropterus dolomieu* Lacépède, 1802) and largemouth bass [*M. salmoides* (Lacépède, 1802)] (Barnhart and Roberts, 1997); M. C. Barnhart (pers. comm.) suspects that spotted bass [*M. punctulatus* (Rafinesque, 1819)] would also serve as a host, although this species has not been tested.

Ptychobranchus occidentalis has experienced the largest reduction in range in Kansas of the five candidate species targeted in this study (Obermeyer *et al.*, 1997). Ten Kansas streams have historic records for this species: the Cottonwood, South Fork of the Cottonwood, Elk, Fall, Caney, Neosho, Spring, and Verdigris Rivers, and Otter (Greenwood County) and Cedar (Chase County) Creeks (Popenoe, 1885; Call, 1885c, 1885d, 1886; Scammon, 1906; Isely, 1924; Cope, 1979, 1985; Metcalf, 1980; Obermeyer *et al.*, 1995, 1997). However, extant representatives have been recovered recently from only four Kansas streams: the Neosho, Spring, Fall, and Verdigris Rivers (Branson, 1966a, 1967; Frazier, 1977; Liechti and Huggins, 1977; Schuster, 1979; Schuster and DuBois, 1979; Cope, 1979, 1983, 1985; Miller, 1993; Obermeyer *et al.*, 1995, 1997). Furthermore, *P. occidentalis* might have recently become extirpated from the Neosho River (Obermeyer *et al.*, 1995). In the Spring River basin, *P. occidentalis* was uncommon. This observation agrees with the contention of Buchanan (1980) and Oesch (1984) that *P. occidentalis*, although widely distributed in the southern half of Missouri, is uncommon at any one locale. Presently, *P. occidentalis* is listed as threatened in Kansas and as a watch species (*i.e.* a species of concern) in Missouri (Anonymous, 1994).

Ptychobranchus occidentalis is a bradytictic breeder (Johnson, 1980; Barnhart and Roberts, 1997) that releases mimetic larval packets from pleated marsupial gills in early spring (Barnhart and Roberts, 1997). The orangethroat [*Etheostoma spectabile* (Agassiz, 1854)], greenside (*E. blennioides* Rafinesque, 1819), yoke (*E. juliae* Meek, 1891), and rainbow darter (*E. caeruleum* Storer, 1845) have been identified as potential hosts (Barnhart and Roberts, 1997). Of these four species, only two species are found in the study area; the greenside darter is found in the Spring River basin, whereas the orangethroat darter is found throughout the study area (Pflieger, 1975; Cross and Collins, 1995).

Cyprogenia aberti occurred historically in Kansas in the Fall, Elk, Verdigris, Neosho, Spring Rivers (Popenoe, 1885; Call, 1885a, b, 1886, 1887a; Scammon, 1906; Murray and Leonard, 1962; Obermeyer *et al.*, 1995, 1997); however, it has been found recently in only the Verdigris, Fall, and Spring Rivers (Branson, 1966b; Liechti and Huggins, 1977; Cope, 1979, 1985; Miller, 1993; Obermeyer *et al.*, 1995, 1997), and is considered rare with a patchy distribution (Obermeyer *et al.*, 1997). Oesch (1984) and Harris and Gordon (1987) reported that in Ozarkian streams of Missouri and Arkansas, *C. aberti* was locally abundant, especially in the Spring (White River system) and Caddo (Ouachita River system) Rivers in Arkansas (Harris and Gordon, 1987). However, we found the species uncommon in the Spring River basin. *C. aberti* is presently listed as endangered in Kansas and as rare in Missouri (Anonymous, 1994).

Gravid females of *Cyprogenia aberti* have been found in all stages of reproductive development throughout the year (Call, 1887b); however, Chamberlain (1934) observed that the species released conglutinates in late winter, and M.C. Barnhart (pers. comm.) observed the periodic release of conglutinates during winter and spring months. Preliminary findings by M. C. Barnhart (pers. comm.) indicate that, from a total of 24 species infected, the banded sculpin [*Cottus carolinae* (Gill, 1861)], log perch [*Percina caprodes* (Rafinesque, 1818)], and fantail darter (*Etheostoma flabellare* Rafinesque, 1819) are suitable hosts, based on Spring River *C. aberti* specimens. Another cited host for *C. aberti* is the goldfish [*Carassius auratus* (Linnaeus, 1758)] (Watters, 1994), based on Chamberlain (1934); however, although glochidial cysts on goldfish were noted for up to five hours following that fish's ingestion of *C. aberti* conglutinates, subsequent examinations of glochidial cysts (*i. e.* after 5 h) were not made to determine host suitability.

Oesch (1984) reported that *Quadrula cylindrica* is restricted in Missouri to the Black, St. Francis, and Spring Rivers; it also occurred historically in Center Creek (Utterback, 1915) as well as in Shoal Creek (Clarke and Obermeyer, 1996). In Kansas, Obermeyer *et al.* (1997) stated that *Q. cylindrica*'s continued persistence in the state is questionable, with extant representatives limited to a few locales in the Spring and Neosho Rivers (Cope, 1985; Obermeyer *et al.*, 1997). Its current distribution contrasts greatly with its past presence in the Neosho, Cottonwood, Spring, Verdigris, and Fall Rivers as well as in Shoal Creek (Popenoe, 1885; Call, 1885b, d; Scammon, 1906; Isely, 1924; Obermeyer *et al.*, 1997). Clarke and Obermeyer (1996) remarked that the species has exhibited a similar trend of decline throughout most of its range in eastern North America. *Q. cylindrica* is presently listed as endangered in both Kansas and Missouri.

Knowledge of the reproductive biology of *Quadrula cylindrica* is based largely on Tennessee populations of *Q. c. strigillata*, except for brief breeding records by Utterback (1915) and Ortmann (1919). Yeager and Neves (1986) found *Q. c. strigillata* to be tachytictic, with the bigeye chub [*Notropis amblops* (Rafinesque, 1820)], spotfin shiner [*Cyprinella spiloptera* (Cope, 1868)], and whitetail shiner [*C. galactura* (Cope, 1868)] potential hosts based on artificial infestations. Although differences between *Q. c. cylindrica* and *Q. c. strigillata* could be due to phenotypic plasticity (Gordon and Layzer, 1989; Clarke and Obermeyer, 1996), it is possible that host specificity varies between eastern populations, especially of *Q. c. strigillata*, and those further west, such as in Kansas and Missouri. Further evidence of host differences is suspected because in the Neosho River, where small populations of this species remain, suitable hosts identified by Yeager and Neves (1986) are believed to be absent (Cross, 1967; F. B. Cross, pers. comm.).

Oesch (1984) described *Alasmidonta marginata* as being widely distributed in the southern half of Missouri, but noted it is uncommon at any one locale. *A. marginata* was first documented in Kansas by Branson (1966a), who found three living specimens in the Spring River in 1964. Although additional specimens have since been collected in the Spring River (Obermeyer *et al.*, 1995), the only other stream record for *A. marginata* in Kansas is from the Marais des Cygnes River in east-central Kansas, based on a recently dead specimen in Franklin County collected in 1983 (Distler and Bleam, 1987) and a relic valve found in Osage County (Obermeyer, 1996b). The recovery of only one living *A. marginata* and the rarity of fresh shell material in the present study raises concern for the species because earlier surveyors (Branson, 1967; Cope, 1985) found the species in the Spring River in Kansas more frequently and at more sites. It is listed in Kansas as endangered, but is not currently listed in Missouri.

Five potential hosts have been identified for *Alasmidonta marginata* (*fide* Howard and Anson, 1923), which is a bradytictic breeder (Ortmann, 1919; Oesch, 1984; Watters, 1994). These are the northern hog sucker [*Hypentelium nigricans* (Lesueur, 1817)], rock bass [*Ambloplites rupestris* (Rafinesque, 1817)], shorthead redhorse [*Moxostoma macrolepidotum* (Lesueur, 1817)], warmouth [*Lepomis gulosus* (Cuvier, 1829)], and white sucker [*Catostomus commersoni* (Lacépède, 1803)], all of which occur in the Spring River basin (Pflieger, 1975; Cross and Collins, 1995).

CONCLUSIONS

Clarke and Obermeyer (1996) rcommended federal listing status for four of the five species targeted in this study; that is, all except *Alasmidonta marginata*, which is on the western edge of its range in the study area and is widely distributed throughout much of eastern North America (Clarke, 1981). Clarke and Obermeyer (1996) stressed the need for habitat conservation as the key element for future protection of these species, and regarded preservation of the Spring River mussel fauna a top priority because of its diverse mussel assemblage. Furthermore, they considered the Spring River a possible refuge from the impending threat of *Dreissena polymorpha* (Pallas, 1771) (French, 1990; Ludyanskiy *et al.*, 1993) because of the rarity of headwater impoundments, which can function as upstream sources for *Dreissena* veligers (McMahon, 1991). They also regarded the Spring River an important refuge because of its tolerance of droughts due to spring-fed flows. We agree that the Spring River is an important resource to concerve, and believe that basin-wide recovery/protection plans [*e. g.* U. S. Fish and Wildlife Service (1994)] should be considered to help protect remaining mussel assemblages in not the Spring River, but in the Neosho, Verdigris, and Fall River basins as well. Because populations of some of these unionids examined in this study could be ecologically distinct despite exhibiting similar shell morphology with other populations (*e. g.* possible differences in host specificity for *Quadrula cylindrica*), unique biological entities are perhaps being overlooked. Therefore, we believe it is important to identify and protect these disjunct and/or distinct populations. We also recommend additional testing of potential fish hosts for these and other species, perhaps on a drainage-by-drainage basis.

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Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment

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Abstract: To develop a suitable diet for rearing recently metamorphosed freshwater mussels, nine species of algae and one live bacterium were tested in various trialgal or bialgal and bacterium combinations. A substratum was included in the culture chambers with each treatment to facilitate pedal-feeding by juvenile mussels. Kaolin, an artificial substratum, and fine sediment were tested in combination with algae and without algae. An unfed (no algae or substratum) control treatment also was tested.

Juveniles fed a freshwater trialgal diet consisting of *Neochloris oleoabundans* Chantanachat and Bold, 1962, *Bracteacoccus grandis* Bischoff and Bold, 1963, *Phaeodactylum tricornutum* Bohlin, 1897, with fine sediment showed the best growth over time (140 d). These individuals achieved a mean shell length of 1747 µm, and had 30.0% survival after 140 d. Other trialgal mixtures containing *N. oleoabundans*, *Nitzschia acicularis* (Kützing) Wm. Smith, 1853, and *Cyclotella meneghiniana* Kützing, 1844, enhanced growth more than a green trialgal mixture of *Chlorella vulgaris* Beyernik, 1890, *Ankistrodesmus falcatus* (Corda) Ralfs, 1848, and *Chlamydomonas reinhardtii* Dangeard, 1888. A diet of fine sediment alone sustained juveniles through 140 d; however, an additional food source such as algae was necessary to increase survival. Bacteria did not contribute appreciably to juvenile growth and survival. Juvenile mussels reared on commercial yeast diets survived only 8 d; juveniles reared on kaolin and algae survived 60 d.

Key Words: juvenile freshwater mussels, algal diets, fine sediment, kaolin

With nearly 70% of native freshwater mussel species (Unionidae) in decline and another dozen commercial species threatened by the invasion of the zebra mussel, research aimed at the conservation of freshwater mussels has become a priority in the United States (Williams *et al.*, 1993). Culture and propagation of freshwater mussels for stock enhancement, preservation of endangered species, and creation of refugia for native populations from the invading zebra mussel, exemplify current conservation efforts. Research reported here is from an ongoing study on the culture and propagation of freshwater mussels. Previous studies in the United States and Europe on culturing freshwater mussels have reported difficulties in maintaining juveniles beyond 4 wk (Lefevre and Curtis, 1910; Howard, 1917, 1922). One reason could be that the food source used to rear juvenile mussels was inadequate. Unfortunately, little information is available on the suitability of various foods for unionids other than the experiments of Coker *et al.* (1921), Imlay and Paige (1972), and Hudson and Isom (1984) who showed that algae, detritus, and commercial fish food (as dissolved nutrients in the water) were potential food sources for freshwater mussels.

Numerous studies in aquaculture have shown that algae are the principal food of marine shellfish (Ukeles, 1971; Webb and Chu, 1983; Tan Tiu *et al.*, 1989), and more importantly, that polyunsaturated fatty acids (PUFA's) are essential to the early larval and juvenile life stages of most fish and shellfish (Ando, 1968; Castell, 1970; Ackman, 1983; Watanabe *et al.*, 1983; Webb and Chu, 1983; Napolitano *et al.*, 1988, 1990). It is generally thought that PUFA's are important functional components of cells and membranes (Ackman, 1983), and that lipid storage products provide a cheap energy source. In addition, mixed diets of different species of algae provided better growth in marine bivalves than did quantities of any single food tested (Davis and Guillard, 1958; Walne, 1970; Epifanio, 1979; Romberger and Epifanio, 1981; Enright *et al.*, 1986a). Resident bacteria in aquatic systems also have been implicated as an important ingredient in the juvenile bivalve diet (Urban and Langdon, 1984; Crosby *et al.*, 1990; Baldwin and Newell, 1991).

Feeding in the sediments with foot ciliation is common among bivalves, especially in the juvenile life stage, until their gills are fully developed for filter-feeding (Allen, 1961, 1985; Morton, 1976; Lasee, 1991; Yeager *et al.*, 1994). Thus, juvenile bivalves can derive nutrition from detrital material adhered to or adsorbed on the surface of sediment particles. In an earlier study, pedal-feeding behavior by juveniles of two freshwater mussel species was reported by Gatenby *et al.* (1996). Specific research objectives of our study, therefore, were to identify a suitable diet for rearing juvenile freshwater mussels, to examine the nutritional role of riverine sediment and bacteria, and to compare growth and survival of juvenile mussels reared on different diets after metamorphosis.

MATERIALS AND METHODS

The rainbow mussel, *Villosa iris* (Lea, 1829), was selected for this study because it is common in Virginia, and like most endangered freshwater mussel species, is found in fast-moving riverine environments. Recently metamorphosed juveniles were fed various combinations of nine species of algae, including six green algae and three diatom species, one bacterium, yeast, kaolin, and riverine sediment (Table 1). Juvenile mussels can contain fatty reserves from their parasitic life stage that can allow them to live 2 wk post-metamorphosis without food supplementation (Lasee, 1991). We decided, therefore, that we would monitor growth and survival in mussels reared on our diets for at least 45 d post-metamorphosis. We were able, however, to maintain juvenile mussels in laboratory culture much longer than 45 d. Thus, three tests were performed on data collected at 60, 100, and 140 d post-metamorphosis. Gut contents of juveniles and river sediment were examined for algae and bacteria. Pedal-feeding behaviors also were observed, and the number of days post-metamorphosis that juveniles employed pedal-feeding was estimated.

Newly metamorphosed mussels were obtained from host-fish infestations in the laboratory (Zale and Neves, 1982; Gatenby *et al.*, 1996). Gravid *Villosa iris* were collected from Copper Creek, Clinch River drainage, Scott County, Virginia. The host fish [rock bass, *Ambloplites rupestris* (Rafinesque, 1817), and largemouth bass, *Micropterus salmoides* (Lacèpéde, 1802)], 15-30 cm in length (Table 1) were collected from Tom's Creek, New River drainage, Montgomery County, Virginia, treated for parasites, and acclimated in the laboratory prior to infestation with glochidia (Zale and Neves, 1982; Neves *et al.*, 1985).

River water and sediment were collected from the New River, Montgomery County, Virginia. Water hardness ($CaCO_3$) was 55.0 mg/l, and pH was 7.6. River water was filtered using a 4.25 cm diameter Whatman Glass Microfiber Filter to remove particles > 0.45 µm. Sediment was maintained in the laboratory under aeration, and when needed, it was sieved through a 130 µm mesh screen before being added to the juvenile culture chamber. Thus, the sieved sediment contained the natural assemblage of microorganisms and other organic material associated with riverine sediments.

Table 1. Summary of diets tested for rearing juvenile freshwater mussels of *Villosa iris*. (CAC = *Chlorella vulgaris, Ankistrodesmus falcatus,* and *Chlamydomonas reinhardtii*; NNiC = *Neochloris oleoabundans, Nitszchia acicularis,* and *Cyclotella meneghiniana*; NOC = *N. oleoabundans, Oocystis marsonii,* and *C. menenghiana*; NPB = *N. oleoabundans, Phaeodactylum tricornutum,* and *Bracteacoccus grandis*; NPE = *N. oleoabundans, P. tricornutum,* and *Enterobacter aerogenes*; Sed = fine sediment).

Treatment (replicates)	Juveniles per replicate
Unfed (1)	45
Kaolin-only (3)	50, 50, 50
Sed-only (2)	71, 70
Yeast/Sed (3)	100, 100, 100
CAC/Kaolin (2)	92, 100
CAC/Sed (3)	200, 200, 215
NOC/Sed (3)	100, 100, 100
NNiC/Sed (3)	111, 100, 103
NPB/Sed (3)	100, 101, 108
NPE/Sed (3)	100, 103, 100

Newly metamorphosed juveniles were transferred to replicated glassculture dishes, 8 cm in diameter, 5 cm in height, and filled with 175 ml of filtered river water. A slow stream of air was introduced into each of the static chambers by fixing an Eppendorf pipette to the end of vinyl air tubing. Approximately 25-40 ml (4 g dry wt) of fine sediment was added to the static chambers, with the exception of those treatments receiving kaolin. Fine sediment covered an area of 50 cm^2, was 1.0-4.0 mm in depth and loosely packed in the culture chamber. Sediment particles we term "fine" were defined as "fine sand to clay" by Wentworth (1922). Approximately 2-3 g of kaolin were added to the kaolin treatments at the onset of the experiment. Water temperature was monitored daily, water was changed weekly, and chambers received new sediment or kaolin weekly. About two-thirds of the substratum in any given culture dish was discarded and replaced with fresh substratum.

Algae were cultured in media ideal for growth of each species (Tables 2, 3, and 4), under continuous cool white fluorescent light of 60-100 µmol/m^2/s photon flux at 20 ± 1°C. All media were autoclaved at 121°C, >15 psi, for 20 min, then cooled prior to being innoculated with algae. Unialgal cultures were not axenic; however, the culture flasks were capped with cotton, cheese-cloth stoppers to prevent contamination and allow aeration through a sterile filter system. At near-maximum stationary phase, algal cells were counted in a hemacytometer. Cultures were then centrifuged at 7000-10,000 rpm for 25 min and the medium decanted. Algae were resuspended in tap water and kept under dark refrigeration for up to 2 wk.

Approximately 1 g of a manipulated yeast diet (Artemia Reference Center, Ghent, Belgium) was mixed in

Table 2. Algae and bacteria species tested in feeding experiment, species sources, and growth medium used to culture species. (BMB = Bold's Modified Bristol's; OCM = Our *Chlorella* Medium).

Species tested	Species source	Growth medium[1]
Neochloris oleoabundans Chantanachat and Bold, 1962	Martek BioSciences Corp., Columbia, Maryland	*Neochloris*[2]
Bracteacoccus grandis Bischoff and Bold, 1963	University of Texas, Austin, Texas	OCM[3]
Ankistrodesmus falcatus (Corda) Ralfs, 1848	Carolina Biological Supply, Burlington, North Carolina	BMB[4]
Chlorella vulgaris Beyernik, 1890	Carolina Biological Supply	BMB[4]
Oocystis marsonii Lemmermann, 1898	University of Texas	OCM[3]
Chlamydomonas reinhardtii Dangeard, 1888	University of Texas	BMB[4]
Cyclotella meneghiniana Kützing, 1844	Loras College, Dubuque, Iowa	Bi-phasic/BMB[4]
Nitzschia acicularis (Kützing) Wm. Smith, 1853	Loras College	Bi-phasic/BMB[4]
Phaeodactylum tricornutum Bohlin, 1897	University of Texas	Soil extract/BMB[4]
Enterobacter aerogenes Kruse, 1896	Biology Dept., Virginia Tech	Nutrient Broth[5]

[1] Media formulations given in Table 3.
[2] McArdle *et al.*, 1994.
[3] Behrens *et al.*, 1989.
[4] Nichols, 1973.
[5] DIFCO, Detroit, Michigan.

500 ml of tap water, the equivalent of 5-10 l of live algae or 1.0×10^7 - 2.0×10^7 cells/ml.

The bacterial culture was axenic and grown in nutrient broth media (Table 2), under continuous 60-100 μmol/m²/s photon flux of white fluorescent light. The air temperature was approximately 20 ± 1°C. Bacterial cell concentration was determined by plate counts. Bacteria were concentrated by centrifugation at 7000 rpm for 20 min; cells were separated from culture media, resuspended in tap water, and then kept alive under refrigeration for the duration of the experiment, with growth arrested at a concentration of 1.0×10^8 cells/ml. Concentrated algae were administered daily to achieve treatment diets of 3.0×10^5 to 5.0×10^5 cells/ml in juvenile culture dishes.

Nine treatments tested whether riverine sediment with associated native bacteria and other organic material provided nutritional value to juvenile mussels, whether bacteria added to the culture diet enhanced growth and survival, and whether various algae or a commercial yeast diet known to be high in lipids, could enhance growth and survival of juvenile mussels. Three replicates of most treatments were tested; however, due to constraints on availability of juveniles, fewer replicates of some treatments were necessary (Table 1). The treatments included an unfed control treatment (Unfed), Kaolin-only (USP K2-500, a fine clay product of Fisher Chemical), fine sediment (Sed-only), yeast with fine sediment (Yeast/Sed), *Chlorella vulgaris, Ankistrodesmus falcatus, Chlamydomonas reinhardtii* with kaolin (CAC/Kaolin), CAC with fine sediment (CAC/Sed), *Neochloris oleoabundans, Oocystis marsonii, C. meneghiniana* with fine sediment (NOC/Sed), *N. oleoabundans, Nitzschia acicularis, Cyclotella meneghiniana* with fine sediment (NNiC/Sed), *N. oleoabundans, Bracteacoccus grandis, Phaeodactylum tricornutum* with fine sediment (NPB/Sed), and *N. oleoabundans, P. tricornutum, Enterobacter aerogenes* with fine sediment (NPE/Sed) (Table 1). Neither sediment nor kaolin was included in the particle concentration calculation; however, the bacterium *E. aerogenes* was included in the particle concentration for the diet NPE/Sed. All experiments were conducted on a 12:12 hr light:dark cycle, and each chamber contained 50-100 juveniles (Table 1).

Table 3. *Neochloris* (McArdle *et al.*, 1994) and OCM (Our *Chlorella* Medium) (Behrens *et al.*, 1989) growth medium formulations.

Neochloris		OCM	
Component	Concentration	Component	Concentration
NaCl	5.82 g/l	KNO_3	1.0 g/l
$MgSO_4 \cdot 7H_2O$	2.47 g/l	$MsSO_4 \cdot 7H_2O$	5.0 ml, 100 g/l
KNO_3	1.0 g/l	$Ca(NO_3)_2 \cdot 4H_2O$	2.5 ml, 25 g/l
KCL	0.75 g/l	K_2HOP_4	2.0 ml, 50 g/l
$FeCl_3$	1.0 ml, 0.81 g/l	K_2HOP_4	1.5 ml, 50 g/l
$CaCl_2$	2.0 ml, 43.9 g/l	Fe - versenate*	2.0 ml/l
Na_2EDTA	2.0 ml, 11.17 g/l	*Na_2EDTA	4 g/l
H_3BO_3	1.0 ml, 12.36 g/l	*$FeSO_4 \cdot 7H_2O$	5 g/l
Metals*	10.0 ml/l	*Distilled H_2O	to 1 l
*$FeCl_3$	20 ml, 33 g/l	Metals*	1.0 ml/l
*$ZnCl_2$	20 ml, 1.1 g/l	**$ZnSO_4 \cdot 7H_2O$	222 mg/ml
*$CuSO_4 \cdot 5H_2O$	2.0 ml, 5 g/l	**$CuSO_4 \cdot 5H_2O$	79 mg/ml
*H_3BO_3	20 ml, 6 g/l	**H_3BO_3	2.86 g/l
*$MnCl_2 \cdot 4H_2O$	20 ml, 16 g/l	**$MnCl_2 \cdot 4H_2O$	1.81 g/l
*$Na_2MoO_4 \cdot 2H_2O$	20 ml, 1.2 g/l	**$Na_2MoO_4 \cdot 2H_2O$	390 mg/ml
*$CoCl_2 \cdot 6H_2O$	2.0 ml, 4 g/l	**$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg/ml
*Na_2EDTA	4.0 g/l	**Distilled H_2O	to 1 l
*Distilled H_2O	to 1 l	Distilled H_2O	to 1 l
Vitamins** (add 50 ml to 1 l media after autoclaving)			
**KH_2PO_4	2.0 ml, 54.4 g/l		
**Thiamine•HCl	0.1 ml, 1 mg/ml		
**Biotin	2.5 ml, 0.2 mg/ml		
**Vitamin B12	2.5 ml, 0.2 mg/ml		
Distilled H_2O	to 1 l		

We examined growth and survival at 60 d and 100 d, and growth over 140 d post-metamorphosis. Juveniles in a culture dish were sieved out and removed to a Petri dish for measuring and assessing survival, weekly. Shell lengths of a random sample of 15 to 25 juveniles in each dish were measured using a calibrated ocular micrometer on a dissecting microscope. Randomness was achieved by moving the Petri dish on the dissecting stage and measuring the first animal that came into view. Juveniles were measured, counted, and returned to their culture dish. Thus, no juvenile was measured twice, nor counted twice.

Shell lengths at 60 and 100 d were compared using one-way analysis of variance (ANOVA) with nested replicates, followed by multiple contrast tests (Sokal and Rohlf, 1981). The survival data were compared by ANOVA (without nested replicates), followed by Tukey-Kramer multiple comparison. Arcsine transformation was applied to the percent survival data to satisfy the normality assumption. Multiple comparisons and contrast tests were evaluated at $\alpha < 0.01$ (0.003 and 0.005 for the 60 and 100 d nested ANOVA's) to control the overall experiment-wise error of $\alpha = 0.05$ (Zar 1974; Sokal and Rohlf, 1981). Growth rates at 140 d post-metamorphosis were compared using analysis of covariance (ANCOVA), followed by a sequential analysis of the slopes to determine differences among treatments. Multiple comparisons were evaluated at $\alpha = 0.01$, so that the overall experimental error also was controlled at $\alpha = 0.05$. Treatments without survivors were not included in any of the analyses. All statistical tests were calculated using JMP 2.0 (SAS Institute, Inc., 1991).

Table 4. Bold's Modified Bristol's (BMB) (Nichols, 1973) and bi-phasic growth media formulations, and soil extract preparation.

Component	Concentration
NaCl	10 ml, 1 g/400 ml
$MgSO_4{\cdot}7H_2O$	10 ml, 3 g/400 ml
$NaNO_3$	10 ml, 3 g/400 ml
K_2HPO_4	10 ml, 3 g/400 ml
KH_2PO_4	10 ml, 7 g/400 ml
$CaCl_2{\cdot}2H_2O$	10 ml, 1 g/400 ml
$Na_2EDTA{\cdot}2H_2O$	1 ml, 50 g/l
KOH	1 ml, 31 g/l
$FeSO_4{\cdot}7H_2O$	1 ml, 5 g/l
H_2SO_4	1 ml, 1 ml/l
H_3BO_3	1 ml, 11.42 g/l
$ZnSO_4{\cdot}7H_2O$	1 ml, 8.82 g/l
$MnCl_2{\cdot}4H_2O$	1 ml, 1.44 g/l
MoO_3	1 ml, 0.71 g/l
$CuSO_4{\cdot}5H_2O$	1 ml, 1.57 g/l
$Co(NO_3)_2{\cdot}6H_2O$	1 ml, 0.49 g/l
Soil extract	40 ml/l
Autoclave soil (with water overlay) two times, at 121°C, > 15 psi, for 20 min. Decant soil water; preferably using Whatman 1 Filter. If still turbid, centrifuge to achieve a clear extract.	
Biphasic	
Place autoclaved river soil to a depth of about 3 cm, with Bold's Basal media overlay.	

Fluorescence microscopy, which induces a distinctive red fluorescence of chlorophyll under blue light, was used to verify that juveniles were ingesting algae, and that the algae were well distributed throughout the substratum. Juveniles were placed on microscope slides and squashed under cover slips for examination.

Locomotory and feeding behaviors of *Villosa iris* were observed using a dissecting microscope at the time of shell measurement and survival tabulation. Approximately 20 juveniles from each culture dish were observed for about 20 min each week.

RESULTS

The Unfed, Kaolin-only, Yeast/Sed, and NOC/Sed diets did not support growth beyond 35-40 d, and thus, were not included in the statistical analyses. Shell lengths of juveniles reared on all other diets were not significantly different ($p = 0.4104$) at 60 d. Shell lengths of juveniles at 100 d also were not significantly different (0.0830); however, all juveniles reared on CAC/Kaolin were dead. Subsequent contrast t-tests at 100 d ($\alpha = 0.005$) indicated that mean shell lengths of juveniles fed NPB/Sed (1354 µm) was significantly greater ($p < 0.0018$) than those of juveniles fed on all other treatments. At 100 d, mean shell lengths of juveniles fed CAC/Sed (874 µm) and Sed-only (828 µm) were similar ($p = 0.6183$), and mean lengths of juveniles fed CAC/Sed, NPE/Sed (978 µm) and NNiC/Sed (1009 µm) also were similar (Table 5). Juveniles fed NNiC/Sed and NPE/Sed, however, exhibited more growth at 100 d than juveniles in Sed-only ($p = 0.0661$ and 0.0556, respectively), although not significant at the contrast test level of $\alpha = 0.005$. Individuals fed the better diet (NPB/Sed) achieved a maximum shell length of 1077 µm at 60 d, and a maximum shell length of 1795 µm at 100 d (Table 5).

An analysis of covariance after 140 d confirmed that juveniles fed various diets exhibited significantly different growth rates over time ($p < 0.001$) (Fig. 1). A sequential analysis of estimated slopes indicated that the growth rate of juveniles fed NPB/Sed was significantly greater than the growth rates recorded for all other treatments, and that all algal diets produced greater growth than a diet of fine sediment only (Table 6). Growth over time was similar for juveniles fed NPE/Sed and NNiC/Sed ($p = 0.7626$), and both diets marginally improved growth over the green algal diet, CAC/Sed ($p = 0.0656$ for NPE/Sed versus CAC/Sed and $p = 0.0177$ for NNiC/Sed versus

Table 5. Mean shell length (± SD, with minimum-maximum in parentheses) and percent survival of *Villosa iris* juveniles fed various diets for 60 and 100 d post-metamorphosis. Initial lengths of juveniles were 263 ± 37 µm. p-values are given for the ANOVA test for treatment effect (α = 0.05). Means with the same superscripts (a, b) were similar according to contrast t-tests and Tukey-Kramer test; however, due to high variability within treatments in survival data, the ANOVA test at 60 d was sufficiently robust to detect differences among treatments. (Abbreviations as in Table 1).

	60 Days		100 Days	
Diet	Length (µm)	Survival (%)	Length (µm)	Survival (%)
Unfed	total mortality		total mortality	
Kaolin-only	total mortality		total mortality	
Sed-only	710^{a} ± 108 (462-897)	50.8	828^{b} ± 75 (667-949)	31.1^{a}
Yeast/Sed	total mortality		total mortality	
CAC/Kaolin	744^{a} ± 69 (641-795)	4.3	total mortality	
CAC/Sed	658^{a} ±108 (500-886)	51.3	874^{b} ± 138 (643-1143)	36.0^{a}
NOC/Sed	total mortality		total mortality	
NNiC/Sed	638^{a} ± 69 (513-769)	13.6	1009^{b} ± 155 (769-1281)	20.7^{a}
NPB/Sed	734^{a} ± 153 (564-1077)	33.7	1354^{a} ± 296 (1026-1795)	32.5^{a}
NPE/Sed	655^{a} ± 105 (513-923)	48.6	978^{b} ± 231 (564-1795)	34.2^{a}
p-value	0.4104	0.4591	0.0830	0.7768

CAC/Sed) (Table 6). After 140 d, individuals fed NPB/Sed achieved a mean length of 1747 µm and maximum length of 2359 µm (Table 7).

Survival at 60 d ranged from 4.4% for CAC/Kaolin to 51.3% for CAC/Sed, and variability within a treatment was high (Table 5). Although 4.4% survival is drastically different from 51.3% survival and is probably different from survival for all other diets, except perhaps NNiC/Sed (13.6%), the ANOVA was of insufficient robustness to detect differences among treatments. Mean percent survival at 100 d for all treatments ranged from 20.7-36.0%, and an ANOVA indicated no difference in percent survival among treatments (p = 0.7768) (Table 5). However, one to two replicates within each treatment showed 0% survival; thus, with so few experimental units, no statistical test was sufficiently robust to adequately analyze our survival data. After 140 d, algae in combination with sediment appeared to gain significance in enhancing survival. Mean survival at 140 d for individuals reared on algae with fine sediment was 24.5%, whereas mean survival for individuals in fine sediment-only was 10% (Table 7).

The Unfed treatment was unable to maintain juveniles beyond 37 d, and the Kaolin-only treatment had no survivors at 38 d. The Yeast/Sed diet did not support juveniles beyond 8 d, and all individuals fed NOC/Sed were dead by 46 d, with replicates 1 and 2 dead at 24 d. Almost all juveniles died in two replicates of NNiC/Sed between 49 and 65 d. Chemical analysis of the prepared diets indicated that NOC and NNiC contained 89.2 and 55.9 mg/l of potassium, respectively, and these levels could have been toxic to mussels (Imlay, 1973). Two tanks of NPB/Sed were lost at 80 d due to a waterline break.

Juveniles were very active during the 20 min they were observed each week. By protracting and retracting the foot, the juveniles moved through the substratum in a "tumble-like" fashion. While lying valve-side down, juveniles continually used a foot-sweeping movement which carried a current of particles toward the pedal gape. The foot was highly ciliated, and some particles adhered to it, while larger particles were lost as the juvenile retracted its foot inside the valves. These behaviors have previously been described by Reid *et al.* (1992) and Yeager *et al.* (1994) for several marine and freshwater bivalves. Fluorescence microscopy indicated that juveniles reared on sediment/algae and kaolin/algae had ingested algae (Fig. 2A), and that the algae were well distributed throughout the substratum (Fig. 2B). Subsequent gut squashes showed par-

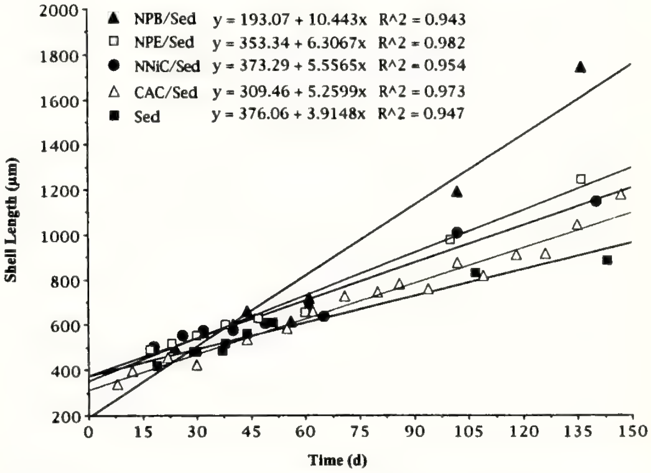

Fig. 1. Comparison of growth equations of juvenile *Villosa iris* fed various diets for 140 d post-metamorphosis. (Abbreviations as in Table 1).

Table 6. Comparison of growth equations of *Villosa iris* juveniles fed various diets for 140 d post-metamorphosis. The diet with the largest slope (NPB) was compared to all other diets first. Diets found to be significantly different from the diet in question were then excluded sequentially from each test until all desired comparisons were made. p-values are given for the multiple comparisons of slope estimates from a sequential analysis of covariance. Slopes are signficantly different at $\alpha < 0.01$, thus, the overall experimental error is controlled at $\alpha = 0.05$. (* = the slope of this diet is significantly different from the diet to which all other diets are being compared in that particular test; na = not applicable; other abbreviations as in Table 1).

		Sequential Comparison of Slopes				
	Growth Equation	NPB/Sed with all other diets	NPB/Sed with NNic/Sed, NPE/Sed	NPE/Sed with all except NPB/Sed	NPE/Sed with NNic/Sed, CAC/Sed	NNic/Sed with, CAC/Sed, Sed-only
NPB/Sed	$y = 193.07 \pm 10.443x$	na	na	na	na	na
NPE/Sed	$y = 353.34 \pm 6.3067x$	0.1729	0.0040*	na	0.7626	na
NNiC/Sed	$y = 373.29 \pm 5.5565x$	0.4952	0.0080*	0.6925	na	na
CAC/Sed	$y = 309.46 \pm 5.2599x$	0.0000*	na	0.1431	0.0656	0.0177
Sed-only	$y = 376.06 \pm 3.9148x$	0.0000*	na	0.0007*	na	0.0045*

Table 7. Mean shell length (± SD, with minimum-maximum in parentheses) and percent survival of *Villosa iris* juveniles in each culture chamber, and pooled survival for all juveniles reared on algae and sediment or sediment-only for 140 d post-metamorphosis. [nd = no data (not considered in the pooled survival calculation); other abbreviations as in Table 1).

Diet	Length (μm)	Survival (%)
Sed-only 1	914 ± 120 (769-1180)	15.5
Sed-only 2	855 ± 74 (769-897)	4.3
Pooled survival for Sed-only		10.0
CAC/Sed 1	nd[3]	nd
CAC/Sed 2	1146 ± 248 (718-1590)	24.5
CAC/Sed 3	nd[3]	nd[3]
NNic/Sed 1	1149 ± 283 (564-1667)	15.3
NNic/Sed 2	nd[1]	nd[1]
NNic/Sed 3	nd[1]	nd[1]
NPB/Sed 1	nd[2]	nd[2]
NPB/Sed 2	nd[2]	nd[2]
NPB/Sed 3	1747 ± 301 (1282-2359)	29.6
NPE/Sed 1	954 ± 249 (610-1439)	30.0
NPE/Sed 2	1369 ± 436 (897-2051)	9.7
NPE/Sed 3	1410 ± 308 (897-2051)	38.0
Pooled survival for algae and sediment		24.5

[1] NNic could have contained toxic levels of potassium (from algal medium).

[2] Burst water line caused mortality.

[3] Chironomid larvae might have preyed upon juveniles.

tially digested algal cells, or ghost cells lacking chloroplasts, as well as colloidal particles, indicating that algae associated with the fine sediment were utilized as food by the juvenile mussels. *Villosa iris* exhibited locomotory and pedal-sweep movements for approximately 140 d. After 272 d, *V. iris* were observed positioned anterior-end in the sediment; apertures were visible and siphoning.

DISCUSSION

The fatty acid composition of many algae has been characterized, although most of the work has focused on marine algae (Ben-Amotz *et al.*, 1985; Cranwell *et al.*, 1988; Ahlgren *et al.*, 1992). Thus, although we did not perform lipid analyses on our diets, it was anticipated that certain freshwater algae high in lipids, especially PUFA's, would enhance growth in juvenile freshwater mussels. Because the culture environment affects the lipid content of algae (Spoehr and Milner, 1949; Fogg, 1959; Shifrin and Chisholm, 1981; Enright *et al.*, 1986b), we harvested our algal cultures at the late-logarithmic, early-stationary phase when algae tend to produce more unsaturated than saturated fatty acids (Chu and Dupuy, 1980; Ahlgren *et al.*, 1992; Dunstan *et al.*, 1993).

Freshwater Chlorophyceae generally lack substantial amounts of long-chained PUFA's and, unlike other marine algae, are able to synthesize only minimal amounts of polyunsaturated fatty acids (Pohl and Zurheide, 1979; Ahlgren *et al.*, 1992). It was not surprising, therefore, that the entirely green algal diet of *Chlorella, Ankistrodesmus,* and *Chlamydomonas* produced poorer growth than the other algal diets tested. It should be noted, however, that these algal species are not without some nutritional value. Total lipids (as percent dry weight) reported for *C. vulgaris, Chlamydomonas* sp., and *Ankistrodesmus* sp. were 13, 20, and 18%, respectively (Shifrin and Chisholm, 1981; Ben-Amotz *et al.*, 1985), and this trialgal mixture produced better growth than a diet of fine sediment only.

We suspected that juvenile mussels fed diets containing the diatoms *Nitzschia, Cyclotella,* and *Phaeodactylum* would show the best growth because

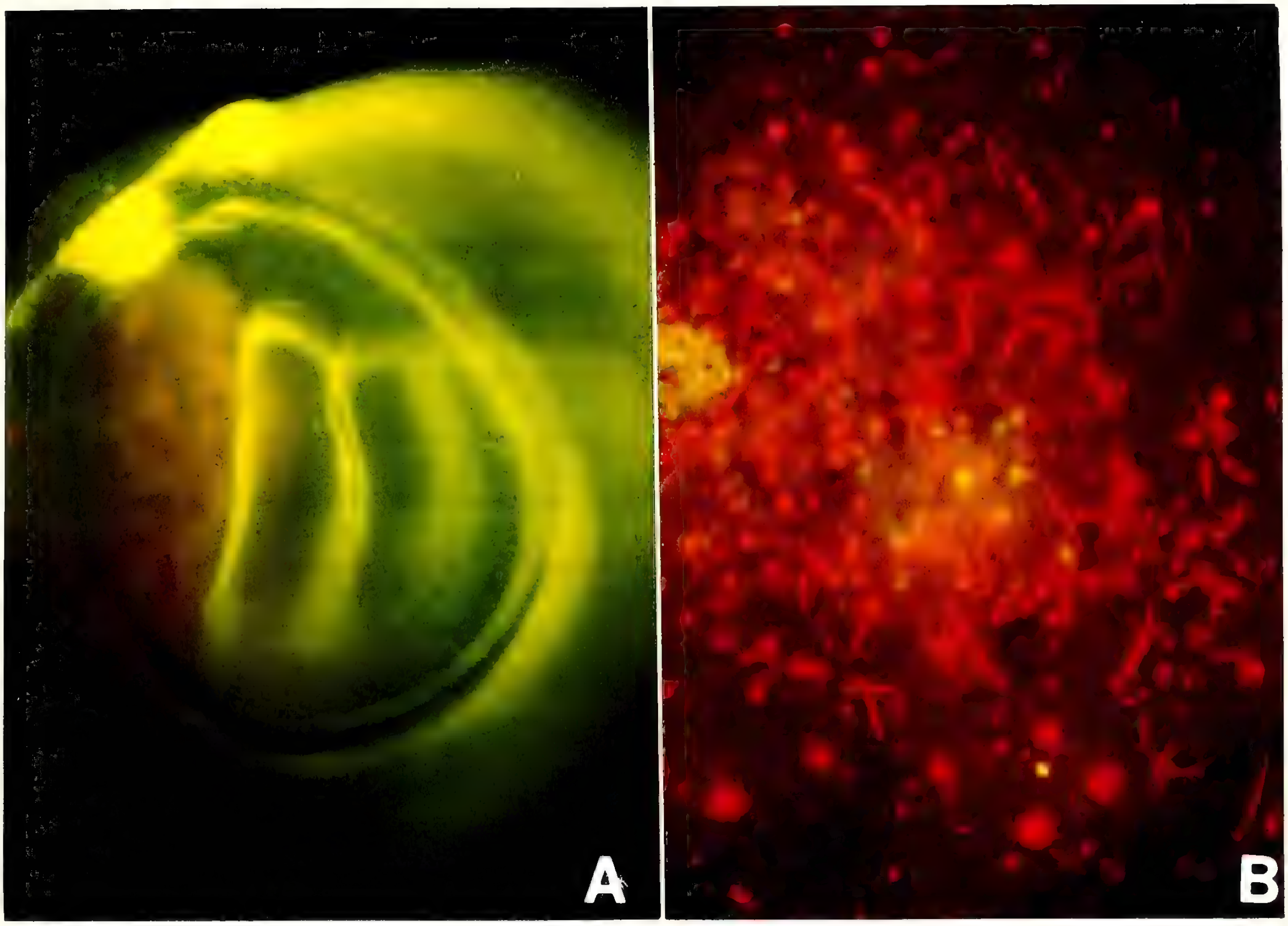

Fig. 2. A. Red fluorescing chlorophyll from ingested algae as seen in gut area of live 30 d juvenile *Villosa iris*. B. Red fluorescing chlorophyll from algae well-distributed in kaolin substratum.

diatoms are characterized by a high percentage of unsaturated fatty acids and generally store oils when nutrients are limited (Erwin, 1973; Werner, 1978; Pohl and Zurheide, 1979; Ahlgren *et al.*, 1992). In addition, species of *Nitzschia* and *Cyclotella* contain more lipids and have a significantly greater proportion of unsaturated fatty acids than green algae (Shifrin and Chisholm, 1981). *P. tricornutum* is abundant in PUFA's (Reitan *et al.*, 1994), and its delicate, siliceous walls are probably loosened easily by grinding or enzymatic action of the digestive system of juvenile mussels. Unlike most green algae, the oil-producing potential of *Neochloris oleoabundans* has been well described (Lien, 1981), and it could also contain PUFA's (Tornabene *et al.*, 1983). *Bracteacoccus grandis,* another green alga, is abundant in lipids (Bishoff and Bold, 1963). Thus, juveniles reared on *Neochloris, Bracteacoccus,* and *Phaeodactylum* showed the best growth over time, and juveniles fed the *Neochloris, Nitzschia,* and *Cyclotella* diet also showed very good growth when compared to those reared under the other diets tested.

Unfortunately, almost all juveniles died in two replicates of NNiC/Sed between 49 and 65 d. This sudden mortality likely resulted from the laboratory culture environment, and not the nutritional quality of the diet. Imlay (1973) reported that exposure of freshwater mussels to potassium levels of 4-7 mg/l over 7 d was lethal. Chemical analysis of the NNiC diet showed high levels of potassium (55.9 mg/l). The algal medium contained high levels of potassium (Tables 3 and 4). Due to the bi-phasic culture methods used to culture *Cyclotella* and *Nitzschia,* soil particles were often extracted along with the algal cells when preparing diets that included these two diatoms. Even though centrifuged cells were rinsed several times, soil particles could have retained more of the algal medium than expected. As judged by the volume of the culture chamber and the volume of algae administered, potassium levels could have reached 6.7 mg/l after 7 d and 9.6 mg/l after 10 d of administering the diet.

All individuals fed NOC/Sed were dead by 46 d, with replicates 1 and 2 dead by 24 d. A chemical analysis of the NOC diet also showed high potassium levels (89.2 mg/l). The water and sediment in each juvenile culture dish was usually changed every 7-10 d. The algae (NOC) were administered daily to the juvenile cultures; however, the water and sediment was not changed during the first 14 d. Thus, the level of potassium in the mussel culture dishes could have reached 21.4 mg/l after 14 d. The causes for high levels of potassium in the diet are possibly two-fold. First, *Oocystis marsonii* is characterized by thick cell walls with large cellulose microfibrils and embedded with hemicelluloses (Preston, 1974). As this was the only alga used in our studies that had such a thick wall, it is possible that more of the algal medium (and excess potassium) was retained by the cells than was desirable. Secondly, soil particles associated with the preparation of this diet also could have retained excessive amounts of the algal medium.

The addition of a cultured live bacterium, *Enterobacter aerogenes,* in combination with the algae *Neochloris* and *Phaeodactylum* and fine sediment, did not enhance growth over the trialgal diets NPB/Sed and NNiC/Sed. This was not surprising because bacteria are generally considered "poor food" for zooplankton due to a lack of PUFA (Wood, 1974). Crosby *et al.* (1990) reported that bacterial mediation enabled the eastern oyster, *Crassostrea virginica* (Gmelin, 1791), to make more efficient use of refractory detrital carbon and nitrogen. Thus, bacteria can break down previously unavailable food sources, but they are not a primary source of nutrition. Juveniles reared in fine sediment only, however, showed a 330% increase in shell length after 140 d. The success of a sediment only diet could be attributed to microbial and organic content because fine sediments contain an array of digestible material (Swain, 1970). Essential minerals, amino acids, and vitamins are also adsorbed onto soil particles creating a storehouse of nutrients (Kelley, 1942; Weiss, 1969; Swain, 1970; Baldwin and Newell, 1991).

Juveniles fed algae with kaolin exhibited growth similar to juveniles fed algae with sediment at 60 d. Thus, resident bacteria in sediments did not enhance growth and are likely not essential to digestion or superior in nutrition to algae. However, survival of juveniles fed algae with kaolin was very low. We attribute this mortality to the fine particle size (1-2 μm) of kaolin. The kaolin became paste-like over time, and we suspect this could have inhibited pedal-feeding or suffocated the juveniles. The fine sediment in our static chambers remained loose in composition and survival of juveniles was high. Yeager *et al.* (1994) observed suspension feeding by juvenile *Villosa iris* in the interstitial spaces of the sediment. More recently, research on the development of gills in juvenile mussels indicates that juveniles filter-feed in combination with pedal-feeding, until gill development is complete (R. Tankersley, pers. comm.). Particle size and composition of the substratum, therefore, seems important for effective pedal-feeding.

The Yeast/Sed diet did not support juveniles beyond 8 d. Preliminary trials using other commercial yeast-based diets also did not support juvenile mussels. These yeast-based diets were developed for mariculture of oysters, clams, and other invertebrates (Coutteau and Sorgeloos, 1992). Because juvenile mussels were short-lived in this treatment, when compared to the sediment only treatment, we believe that this diet could contain a toxic component that is otherwise not toxic to marine organisms. Perhaps the commercial yeast diet will be suitable if used in concentrations lower than that used in our experiments.

Although sediment provides minerals and some organic food material, an additional food source such as algae increased survival and growth. No specific algal diet produced statistically better survival over another. Thus, many algal diets administered with fine sediment could support juvenile mussels for at least several months. For long-term propagation, the more nutritious algae should produce the best growth and survival.

Information on the early life history requirements of freshwater mussels is minimal, with their nutritional requirements probably the least understood. Early investigators believed that "finding suitable nutrition for the first month or so of free life" was critical to the success of rearing mussels (Lefevre and Curtis, 1910, 1912; Coker *et al.*, 1921; Howard, 1922). This study has shown that the trialgal mixture of *Neochloris oleoabundans, Phaeodactylum tricornutum,* and *Bracteacoccus grandis* was a suitable diet for rearing juvenile mussels, and provided adequate nutrition for growth and survival through 140 d post-metamorphosis. The algae *Nitszchia acicularis,* and *Cyclotella meneghiniana* in combination with *N. oleoabundans* also was suitable for rearing juvenile mussels to 140 d post-metamorphosis. However, juveniles reared on *N. oleabundans, P. tricornutum,* and *B. grandis* showed the best growth over time. In addition, adult broodstock of various freshwater species have been maintained on *N. oleoabundans* to 10 mo at Virginia Tech's Aquaculture Center (F. O'Beirn, pers. comm.).

Research in aquaculture indicates that fish and marine bivalves have different requirements for protein, carbohydrates, and polyunsaturated fatty acids as they mature (Walne, 1973; Watanabe *et al.*, 1983; Hawkins and Bayne, 1991). It is likely, therefore, that certain algae might be more suitable to enhance growth in juvenile mussels, and other algae are more suitable for growth of adult mussels. The future success of propagating freshwater mussels will depend on a better understanding of the feeding ecology and environmental requirements of the juvenile life stage.

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Zebra mussel induced mortality of unionids in firm substrata of western Lake Erie and a habitat for survival

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Abstract: The present study was conducted to determine impacts of zebra mussel [*Dreissena polymorpha* (Pallas, 1771); Dreissenidae] infestation on unionids in firm substrata in western Lake Erie. Unionid mollusks were collected at a total of 15 stations on three offshore depth contours (2, 3, and 4 m) in 1983 (before zebra mussel infestation), in 1990 and 1993 (after zebra mussel infestation), and at one station on a nearshore 2-m depth contour and along one transect on a nearshore 1-m depth contour in 1993. Numbers of living unionids on substrata along offshore contours remained similar between 1983 and 1990 and then decreased from 97 individuals in 1990 to only five individuals in 1993. In addition, the number of species decreased from nine to four between 1990 and 1993. In contrast, on nearshore contours 85 living individuals representing nine species were found in 1993. About 48% of the living and 79% of the dead unionids at the two nearshore locations were covered with byssal threads of dreissenid mussels, but were not actively infested by mussels. The presence of living unionids on nearshore contours of western Lake Erie in 1993 indicates that survival of unionids in the presence of abundant zebra mussel populations can be possible in firm substrata and that these habitats can provide natural "refugia" for unionid populations. At present, we do not know what allows unionids to survive in the presence of zebra mussel colonization, but believe that water-level fluctuations and waves could contribute to the removal of mussels from unionids. This information could be of major concern in the mitigation of impacts of infestation on unionids in waters throughout North America.

Key words: *Dreissena*, Unionidae, refugia, Great Lakes, unionid mortality

Zebra mussels [*Dreissena polymorpha* (Pallas, 1771); Dreissenidae] have been shown to be ectoparasites causing reduced fitness, shell deformities, and mortality of unionids (Bivalvia: Unionidae) in waters of Europe and North America (Ricciardi *et al.,* 1995; Schloesser *et al.,* 1996). In the Laurentian Great Lakes, infestation of unionids by zebra mussels was one of the first and most visible ecological impacts of the early invasion of mussels into North America (Hebert *et al.*, 1989, 1991; Mackie, 1991; Schloesser and Kovalak, 1991; Hunter and Bailey, 1992; Haag *et al.,* 1993; Nalepa and Schloesser, 1993). Unionid mortality increased as a result of infestation and unionid populations were nearly eliminated in some areas (Gillis and Mackie, 1994; Schloesser and Nalepa, 1994; Nalepa *et al.*, 1996). In addition, zebra mussels have invaded and are believed to be causing unionid mortality in major rivers of North America, such as the Detroit, St. Lawrence, Mississippi, Ohio, and Tennessee Rivers (Tucker, 1994; Ricciardi *et al.*, 1996; Strayer and Smith, 1996; reviewed by Schloesser *et al.*, 1996).

In western Lake Erie, increased mortality of unionids attributed to zebra mussel infestation has only been documented in soft-mud substrata of open waters (> 6 m deep) (Schloesser and Nalepa, 1994). Soft substrata are believed to suffocate unionids that cannot maintain themselves at the substrate-water interface due to the added weight of infesting zebra mussels (Schloesser and Nalepa, 1994). However, unionid mortality caused by infestation has occurred in some firm-substratum habitats (*e. g.* sand and gravel) where suffocation caused by sediments is unlikely (Nalepa, 1994; Tucker, 1994; Schloesser *et al.*, 1996), but observations indicate that unionids do survive in the presence of zebra mussels on some firm substrata (DWS: unpub. data; D. Blodgett, Illinois Natural History Survey, Havana, Illinois, pers. comm.).

The present study was performed to determine changes in the unionid population in firm compacted sand substrata along the perimeter of western Lake Erie in 1983, before zebra mussels were present, and 1991 and 1993, after mussels were present. We hypothesized that if smothering in soft substrata is the only mechanism causing unionid mortality in western Lake Erie, then unionids should survive infestation in firm substrata along the perimeter of western Lake Erie.

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METHODS

Unionid bivalve mollusks and infesting zebra mussels were collected at 15 stations located on three offshore depth contours (2, 3, and 4 m depths) along the perimeter of western Lake Erie between 23 August and 15 October 1983, 31 October and 11 November 1990, and 8 September and 27 October 1993 (Fig. 1). Unionids were also collected at one station on a nearshore 2-m depth contour, 9 September 1993 and in a transect area on a nearshore 1-m depth contour, 21 October 1993. The offshore 2-m and nearshore 2-m depth contours were separated by a deeper 3-m contour. Fluctuations of daily water levels were corrected based on the frame of reference of low water data at Father Point, Quebec (Great Lakes Basin Commission, 1975). In general, sampling depths were about 1 m deeper than low water datum, except for the period of time when the nearshore transect was sampled during a seiche. All living and dead unionids (represented by both valves) were collected and used in the present study. In addition, total counts of valves and shell pieces of unionids were determined in 1990 and 1993.

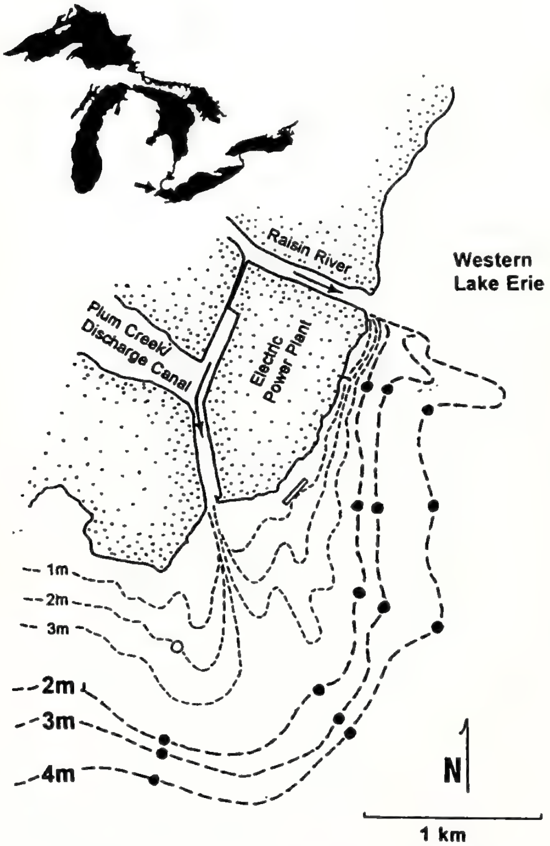

Fig. 1. Locations in western Lake Erie where unionid mollusks were sampled: at 15 stations (●) on three offshore depth contours (dark dashed lines; 2-, 3-, and 4-m) in 1983, 1990, and 1993, and at one station (○) on a nearshore 2-m depth contour (light-dashed line) and along one transect (☐) on the nearshore 1-m depth contour (light-dashed line) in 1993.

Unionids at stations on offshore contours and the nearshore 2-m depth contour were collected by SCUBA (Fig. 1). At each station, random searching was conducted for 30 min within a 50-m diameter circle. Unionids were removed from the water, individually separated, and taken to the laboratory.

Unionids in the transect area (7 m by 150 m) along the nearshore 1-m depth contour were collected by walking and manually removing exposed specimens (Fig. 1). This was possible because water in western Lake Erie was pushed away from shore by westerly winds causing a seiche, thereby dewatering substrata on the nearshore 1-m depth contour for a period of about 6 hr.

In the laboratory, up to ten randomly selected unionids and attached zebra mussels from one station on the offshore 4-m depth contour, the station on the nearshore 2-m depth contour, and the transect on the nearshore 1-m depth contour were analyzed. Living infested unionids were selected, except from the offshore, 4-m depth contour in 1993 when only dead unionids were present. Schloesser and Nalepa (1994) have shown that length-frequency distributions of zebra mussels from living and dead unionids in western Lake Erie are similar. Infesting zebra mussels were removed from individual host unionids and retained in a U. S. Standard Number 60 sieve (0.25-mm mesh). Length-frequency distributions of infesting zebra mussels were constructed from shell-length measurements in 1-m size classes. All mussels or a randomly selected sub-sample of 200-300 mussels (< 6-mm long per unionid) and all mussels > 7-mm long were measured in each sampling period. All mussels were identified and counted. All mussels were zebra mussels (*Dreissena polymorpha*); no quagga mussels (*Dreissena bugensis* Andrusov, 1897) were present in western Lake Erie (Mills *et al.*, 1993; MacIsaac, 1994). Length-frequency distributions of unmeasured mussels < 6-mm long were based on the proportions of measured sub-sampled mussels in each whole millimeter size group. This procedure has been shown to adequately determine length-frequency distributions of zebra mussels in western Lake Erie (Schloesser and Kovalak, 1991; Schloesser and Nalepa, 1994). Dry weights (in g at 105°C for 48 hr) of individual unionids and infesting mussels (a measure of infestation intensity; Schloesser and Kovalak, 1991) were determined.

Unionids were identified following Clarke (1981) and by comparison with bivalve taxonomic reference collections (Detroit Edison Company, Detroit, Michigan, and Great Lakes Science Center, Ann Arbor, Michigan). Taxonomic nomenclature follows Williams *et al.* (1993) with the exception that *Lampsilis radiata radiata* (Gmelin, 1791) was combined with *L. siliquoidea* because these two species are believed to interbreed in the Great Lakes

(Clarke, 1981). Mean numbers of living and dead unionids per station at 15 stations on the three offshore depth contours were tested by Student's t-test after $\log_{(10)} + 1$ transformation (Snedecor and Cockran, 1967).

RESULTS

UNIONIDS ON OFFSHORE CONTOURS

Numbers of living and dead unionids changed substantially on the three offshore depth contours in western Lake Erie between 1983 and 1993 (Table 1). In 1983, total mean number of living unionids per station was significantly greater (paired t-tests, $P \leq 0.05$) than dead unionids. In 1990 and 1993, total mean numbers of dead unionids were significantly higher than living unionids. Numbers of living unionids remained about the same between 1983 and 1990: 85 and 97 individuals, respectively. Then they decreased significantly from 97 in 1990 to only five in 1993. Numbers of dead unionids increased significantly from nine in 1983 to 360 in 1990, then decreased to 157 in 1993. In 1983, mean numbers of living individuals were significantly greater than numbers of dead individuals on the 2-m and 4-m depth contours; in 1990, there were significantly more numbers of dead than living individuals on the 3-m and 4-m depth contours. In 1993, the numbers of dead individuals was greater than the number of living on all contours. Between 1983 and 1990, the number of living unionids increased on the 2-m and 3-m depth contours and decreased on the 4-m depth contour. The greatest increase in the number of dead unionids occurred on the 4-m depth contour between 1983 and 1990.

Numbers of living unionid species decreased on offshore depth contours in western Lake Erie between 1983 and 1993 (Table 2). Twelve living species were found in 1983, nine species in 1990, and four species in 1993. Between 1983 and 1990, the number of species on the 2-m and 3-m depth contours increased from seven to nine, whereas the number on the 4-m depth contour decreased from 12 to six species. In 1993, only four species (*Amblema plicata plicata, Lampsilis siliquoidea, Potamilus alatus,* and *Quadrula quadrula*) represented by five individuals were found on the 2-m and 3-m depth contours.

Table 1. Numbers of living and dead unionids collected at 15 stations on three offshore depth contours in western Lake Erie, 1983, 1990, and 1993. (*; significant difference [$P \leq 0.05$] in mean number of unionids per station).

Depth (m)	1983 Living		1983 Dead	1990 Living		1990 Dead	1993 Living		1993 Dead
2	19	*	1	31		26	2		20
3	12		3	27	*	59	3		41
4	54	*	5	39	*	[1]275	0	*	96
Total	85	*	9	97	*	360	5	*	157

[1]significant ($P = 0.060$).

UNIONIDS ON NEARSHORE CONTOURS

Relatively large numbers of living unionids occurred in sampled areas on the nearshore 2-m and 1-m depth contours (Table 3). A total of 85 living unionids represented by nine species occurred at nearshore locations. Only living unionids (55 individuals, represented by eight species) were collected at the nearshore 2-m depth station. Both living (30 individuals, represented by five species) and dead (29 individuals, represented by ten species) unionids were found in the transect area on the nearshore 1-m depth contour.

Only 15% (17 of 114 individuals) of the unionid shells collected on nearshore contours were infested with zebra mussels (Table 3). In addition, 70% (80 of 114) only showed evidence of past infestation (*i. e.* only byssal threads present on unionid shells). Evidence of infestation was absent on 15% (17) of individual shells (10% [11] of these were living and 5% [6] were dead unionids).

INFESTING ZEBRA MUSSELS

Mean dry weights of infesting zebra mussels were substantially higher on the offshore 4-m depth contour than on the nearshore 2-m depth contour; no infestation occurred on the 1-m nearshore depth contour (Table 4). On the offshore contour in 1990, mean infestation weights on living and dead unionids were 41.1 and 36.3 g/unionid, respectively; in 1993, infestation weights decreased to 32.9 and 27.6 g/unionid, respectively. In general, weights of infesting zebra mussels were about equal to host unionid weights on the offshore contour. In nearshore areas, weights of infesting zebra mussels ranged between 0.5 and 4.4 g/unionid, and mean infestation weights were substantially less than mean unionid weights.

Length-frequency distributions of zebra mussels removed from unionids collected on the offshore 4-m depth contour and the nearshore 2-m depth contour in Fall 1993 were substantially different (Fig. 2). Mussels on the offshore contour were larger (mean = 12 mm long) than those on the nearshore contour (mean = 6 mm long). Peak distribution of mussels on unionids from the offshore contour was between 12 and 16 mm, whereas the peak distribution of mussels on the nearshore contour was between 3 and 7 mm.

DISCUSSION

UNIONID MORTALITY ON OFFSHORE CONTOURS

The present study, that by Nalepa (1994) in Lake St. Clair immediately upstream of western Lake Erie, observations by Tucker (1994) in the Mississippi River, and data of

Table 2. Numbers of living unionids collected at 15 stations on three offshore depth contours in western Lake Erie, 1983, 1990, and 1993. (–, none collected).

Species	1983 Depth (m)			1990 Depth (m)			1993 Depth (m)		
	2	3	4	2	3	4	2	3	4
Amblema plicata plicata (Say, 1817)	1	1	2	12	3	12	–	2	–
Elliptio dilata (Rafinesque, 1820)	–	–	1	–	–	–	–	–	–
Fusconaia flava (Rafinesque, 1820)	2	1	16	1	2	8	–	–	–
Lampsilis ovata (Say, 1817)	–	–	2	–	1	–	–	–	–
L. siliquoidea (Barnes, 1823)	6	6	14	7	16	9	1	–	–
Leptodea fragilis (Rafinesque, 1820)	1	2	1	3	1	1	–	–	–
Ligumia recta (Lamarck, 1819)	–	–	1	–	–	–	–	–	–
Obliquaria reflexa (Rafinesque, 1820)	1	–	1	2	–	1	–	–	–
Potamilus alatus (Say, 1817)	–	–	2	–	–	–	1	–	–
Pyganodon grandis (Say, 1829)	–	–	6	–	1	–	–	–	–
Quadrula pustulosa pustulosa (Lea, 1831)	6	1	7	5	1	8	–	–	–
Q. quadrula (Rafinesque, 1820)	2	1	1	1	2	–	–	1	–
Total Species	7	6	12	7	8	6	2	2	0

Schloesser (unpub. data) indicate that high unionid mortality can occur on firm substrata where smothering by soft substrata is unlikely. In addition, recent data indicate that high unionid mortality could be occurring in areas where infestation does not exist, but zebra mussels are found on surrounding substrata (Ricciardi *et al.*, 1996; Strayer and Smith, 1996).

In the present study, zebra mussel infestation caused substantial unionid mortality (94%) in firm-sand substrata located on offshore contours of western Lake Erie between 1983 and 1993. Mortality occurred between 1990 and 1993 after five years (1989-1993) of zebra mussel colonization of nearshore waters; not between 1983 and 1990 (after one year of zebra mussel colonization) when densities of unionids actually increased. In the fall of 1989, zebra mussels became very abundant in western Lake Erie reaching densities in excess of 340,000/m^2 in open waters (> 6 m depth) and 700,000/m^2 in nearshore waters (2-3 m depth) and infestation intensities in excess of 10,000/unionid (Schloesser and Kovalak, 1991; Kovalak *et al.*, 1993;

Table 3. Numbers of living and dead unionid species with attached zebra mussels and with attached byssal threads of zebra mussels collected at one station on a nearshore 2-m depth contour, 8 September 1993, and in one transect area on a nearshore 1-m depth contour, 21 October 1993, in western Lake Erie.

	Station on Nearshore 2-m Depth Contour[1]			Transect on Nearshore 1-m Depth Contour[2]			
	Living Unionids			Living Unionids		Dead Unionids	
	Attached Zebra Mussels	Byssal Threads		Byssal Threads		Byssal Threads	
		Present	Absent	Present	Absent	Present	Absent
Amblema plicata plicata (Say, 1817)	13	12	2	13	3	8	–
Fusconaia flava (Rafinesque, 1820)	1	2	–	2	2	1	1
Lampsilis ovata (Say, 1817)	–	–	–	–	–	1	–
L. siliquoidea (Barnes, 1823)	–	1	–	1	–	2	2
Leptodea fragilis (Rafinesque, 1820)	–	1	–	–	–	2	–
Obliquaria reflexa (Rafinesque, 1820)	–	1	–	–	–	1	–
Pleurobema cordatum (Rafinesque, 1820)	–	1	1	–	–	–	–
Potamilus alatus (Say, 1817)	1	7	–	–	–	3	1
Pyganodon grandis (Say, 1829)	–	–	–	–	–	2	2
Quadrula pustulosa pustulosa (Lea, 1831)	2	7	1	3	1	2	–
Q. quadrula (Rafinesque, 1820)	–	3	–	4	1	1	–
Total Number	17	34	4	23	7	23	6
Total Species	4	8	3	5	4	10	4

[1] No dead unionids.

[2] No attached zebra mussels.

Leach, 1993; Schloesser and Nalepa, 1994). To date, mortality of unionids induced by abundant densities of zebra mussels has been documented in Europe in the mid-1930s, waters of the Great Lakes, the Hudson River and, possibly, the Mississippi River in the early-1990s (Sebestyen, 1938; Gillis and Mackie, 1994; Schloesser and Nalepa, 1994; Tucker, 1994; Nalepa *et al.*, 1996; reviewed by Schloesser *et al.*, 1996; Ricciardi *et al.*, 1996; Strayer and Smith, 1996; DWS, unpub. data).

Changes in the number of dead unionids in the study area are partly attributable to the movement of dead shells into and out of the study area. The large increase in the number of dead unionids (nine to 360) between 1983 and 1990 suggests that dead unionid shells were entering the sampled area from other areas of western Lake Erie prior to 1990. Schloesser and Nalepa (1994) have shown that at one station in open waters (> 6m deep) of western Lake Erie, mortality of infested unionids increased between Fall 1989 and Spring 1990, and by Fall 1990 was 100%. This period of time corresponds with the large increase in the number of dead unionids in the study area, especially on the offshore, 4-m depth contour in Fall 1990. In 1990, most shells appeared fresh-dead; some contained decaying tissues, many exhibited pearly nacre, and few were decalcified. In 1993, however, few shells appeared fresh-dead; no decaying tissues were found, few exhibited a pearly nacre, and most were decalcified and difficult to identify. Loss of pearly nacre is believed to occur within 6-12 mo after death (Schloesser and Nalepa, 1994; D. Neves, National Biological Service, Blacksburg, Virginia, pers. comm.). In addition, less than 5% of the valves (n = 252) collected in 1990 were described as pieces, whereas about 25% of the valves (n = 256) collected in 1993 were described as pieces. The decrease in the number of dead unionids between 1990 and 1993 is attributed to transport of shells out of the study area into shallow bays of the lake. Substrata in shallow water bays near the sampling area were largely covered with shells of dead unionids in 1993 and 1994 (DWS, GDL, RS, pers. obs.). Several of these areas (50 m by 1000 m transects) were described as "union-

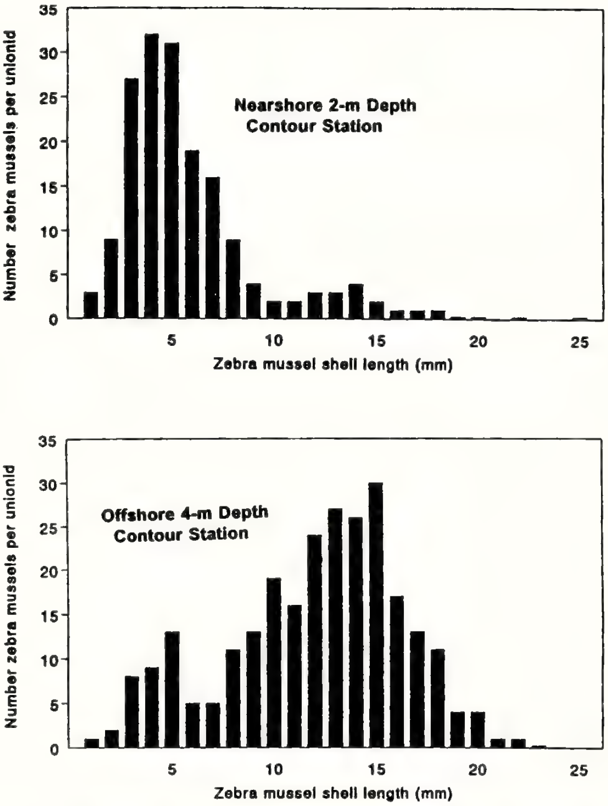

Fig. 2 . Length-frequency distributions of zebra mussels infesting unionid mollusks collected on the nearshore 2-m depth contour and the most western station on the offshore 4-m depth contour in western Lake Erie, 8 September 1993.

Table 4. Mean (± S.E. per unionid) and range of dry weights (g) of infesting zebra mussels and, in parentheses, mean (± S.E.) and range of dry weights of unionids at stations on offshore and nearshore depth contours in western Lake Erie 1990 and 1993.

Location/Date	Living Unionids	Dead Unionids
Offshore 4-m depth contour 1 November 1990[1]	n = 10[2] 41.1 ± 2.9 29.7 - 61.2 (50.5 ± 9.2) (21.8 - 119.9)	n = 10 36.3 ± 4.9 17.3 - 67.3 (46.1 ± 3.1) (29.0 - 62.1)
8 September 1993	n = 5[3] 32.9 ± 7.6 6.8 - 49.0 (39.8 ± 18.0) (32.8 - 118.1)	n = 10 27.6 ± 2.8 15.1 - 40.3 (73.3 ± 10.5) (27.5 - 123.0)
Nearshore 2-m depth contour 9 September 1993	n = 10 4.4 ± 2.0 0.5 - 19.0 (106.9 ± 12.4) (61.7 - 188.0)	n = 0
Nearshore 1-m depth contour 21 October 1993	n = 30 0 0 (85.7 ± 8.0) (28.4 - 158.5)	n = 29 0 0 (79.8 ± 7.2) (24.4 - 164.6)

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[1] Most southwestern station (Fig. 1, open circle) on offshore, 4-m depth contour.

[2] Number of unionids.

[3] Offshore 2-m and 3-m depth contour; no living unionids along the offshore 4-m depth contour.

id graveyards" with a paved-like bottom of unionid shells. Large accumulations of dead unionids has rarely been seen in the Great Lakes prior to colonization by zebra mussels (Neves, 1987; WPK, Michigan, pers. obs.).

Survival of a few unionids on offshore contours in western Lake Erie supports the data of Schloesser and Nalepa (1994) and Nalepa *et al.* (1996) that infestation on unionids by zebra mussels does not cause 100% mortality of all unionids, but does reduce the population to < 5% of pre-zebra mussel colonization. The survival of a few unionids along the offshore 2-m and 3-m depth contours (five living of 157 total unionids) in 1993 is similar to that (four living of 191 total unionids) found in open waters of western Lake Erie in 1991 (Schloesser and Nalepa, 1994). However, the long-term viability of unionid populations at low densities has been questioned, even in the absence of infestation (Lefevre and Curtis, 1910; Downing and Downing, 1992; Downing *et al.*, 1993). To date, artificial maintenance of infested unionids has shown that survival of individual unionids in waters of western Lake Erie is possible (Schloesser, 1996).

Mean weights of zebra mussels infesting unionids on offshore contours (27.6-41.1 g/unionid) were similar to weights of infesting mussels that have caused nearly 100% mortality of unionids in other studies in western Lake Erie (Schloesser and Kovalak, 1991; Schloesser and Nalepa, 1994; GDL and RS, pers. obs.). In 1989, immediately after zebra mussels increased exponentially in western Lake Erie, weights of infesting mussels were between 30.0 and 54.9 g/unionid in nearshore waters and between 9.0 and 75.3 g/unionid in open waters (Schloesser and Kovalak, 1991; Schloesser and Nalepa, 1994). In 1990, weights in open waters ranged from 2.3 to 40.8 g/unionid (Schloesser and Nalepa, 1994). These data indicate that weights of infestation that equal or exceed host unionid weights cause severe mortality of unionids in western Lake Erie (similarly noted as mean:mass ratios by Ricciardi *et al.*, 1996).

UNIONID SURVIVAL IN NEARSHORE WATERS

Relatively large numbers of living infested unionids and living unionids showing evidence of past infestation on nearshore-depth contours indicates that zebra mussel induced mortality of unionids could be minimal or does not exist in some areas along the perimeter of western Lake Erie. The high incidence of infestation (90%) of living unionids on nearshore contours indicates that infestation did occur and that mortality of unionids could have occurred. However, temporal variation in the number of mussels per unionid and densities of mussels colonizing adjacent bottom substrata were not assessed in the present study, and these factors are believed to influence zebra mussel induced mortality of unionids (Schloesser and Kovalak, 1991; Ricciardi *et al.*, 1995; Schloesser *et al.*, 1996).

At present, the reason unionids survive in the nearshore area of western Lake Erie is not known. Several studies have suggested that some species of unionids are more likely to survive zebra mussel infestation than other species (Haag *et al.*, 1993; Nalepa, 1994; Gillis and Mackie, 1994; Tucker, 1994). Possible explanations for these observations include; unionid sex (males less impacted), robustness of shells (unionid species with robust shells less impacted), and length of brooding time (unionid species with short brooding periods less impacted) (reviewed by Schloesser *et al.*, 1996). In the present study, robust species with short brooding times (*Amblema plicata plicata, Fusconaia flava, Quadrula pustulosa pustulosa, Q. quadrula*) accounted for about one-half (57%) the individuals on offshore contours in 1990. By 1993, nearly all unionid individuals were extirpated from offshore contours and of those individuals remaining, three of five were robust, short-term brooder species. However, robust species with short brooding times (four above and *Pleurobema cordatum*) accounted for 87% of living unionids on nearshore contours in 1993. These data support the belief that robust shelled, short-term brooders appear to survive longer than thin-shelled, long-term brooders. But, to date, a total of 31 species of unionids have been infested by zebra mussels in North America, and none appear to be immune to impacts (reviewed by Schloesser *et al.*, 1996).

Possible explanations for survival of unionids in shallow waters of western Lake Erie is that infesting zebra mussels could be removed from unionids and surrounding substrata by predators such as fish and ducks, or mussels could release from substrata in response to unfavorable habitat conditions (Stanczykowska, 1977; French and Bur, 1993; Mitchell and Carlson, 1993; Stanczykowska and Lewandowski, 1993; Hazlett, 1994).

Length-frequency distributions of zebra mussels infesting unionids and SCUBA observations from offshore and nearshore areas indicate that removal of mussels in the nearshore area by predators is not the likely cause for low infestation of unionids in the nearshore area. Length-frequency distributions indicate that mostly small, young mussels were present on unionids in the nearshore area, whereas mostly larger, older mussels were present in the offshore area. This indicates that mussels probably leave unionids between their first and second year of life because peak modes often correspond to year-classes of mussels (Morton, 1969; Stanczykowska, 1977; Schloesser and Kovalak, 1991) and typically, length-frequency distributions of zebra mussels in Fall in western Lake Erie are bimodal (Griffiths *et al.*, 1991; Schloesser and Kovalak, 1991; Schloesser and Nalepa, 1994). In addition, similar observation by SCUBA indicate that zebra mussels attached to substrata in the nearshore area were of low density and small size compared to mussels in the offshore area (GDL and RS, pers. obs.). Because mussels have been shown to have great adhesive strength and predators tend to remove smaller mussels rather than larger mussels (Ackerman *et al.*, 1993; Eckroat *et al.*, 1993; French and Bur, 1993; Hamilton *et al.*, 1994), we believe that mussels were not removed by predators in the nearshore area. Rather, zebra mussels voluntarily released from unionids, perhaps because of factors such as waves, water-level fluctuations, and ice scour in nearshore areas (< 2-m depth). In Lake Erie, mortality of unionids has occurred in another nearshore area (2-3 m depth) where no waves, exposure due to water-level fluctuations, or ice scour occur (Schloesser and Kovalak, 1991; Schloesser 1996; WPK, unpub. data). Movement *en masse* of zebra mussels has been observed and attributed to fall storms, wave action, and ice scour in Europe and western Lake Erie (Ehrenberg, 1957; Lewandowski, 1976; Griffiths *et al.*, 1991; Nalepa and Schloesser, 1993). In the Illinois River, Tucker and Atwood (1995) suggested that changing water levels (among other factors) could have contributed to substantial decreases in numbers of zebra mussels in an area.

Areas where unionids survive in the presence of zebra mussel colonization appear to be natural "refugia" where long-term survival of unionids could be possible. Although long-term studies have not been completed, several sites have been found where unionids continue to live in the presence of zebra mussel colonization and large year-to-year fluctuations in the density of mussels in surrounding areas (Tucker and Atwood, 1995; Schloesser *et al.*, 1996; DWS, unpub. data; D. Blodgett, Illinois Natural History Survey, Havana, Illinois, and D. Miller, U. S. Army Corps of Engineers, Vicksburg, Mississippi, pers. comm.). Observations such as these lead Clarke, (1992), Masteller *et al.*, (1993), Tucker and Atwood (1995), and Schloesser (1996 and unpub. data) to identify possible refugia and/or suggest the need for the establishment of managed refugia to save unionid populations. These refugia would only be needed in a few areas where unique unionid species and populations are found because many waters in North America are unlikely to be heavily colonized by zebra mussels (Strayer, 1991). Indeed, dreissenid mussels in rivers appear to be a threat to unionids immediately below impoundments and lakes that provide mussel veligers contributing to infestation (Schloesser *et al.*, unpub. data; DWS and D. Hunter, Oakland University, Rochester, Michigan, unpub. data).

CONCLUSIONS

Results of the present study, conducted in firm-substratum areas along the perimeter of western Lake Erie, were similar to those of Schloesser and Nalepa (1994) who studied soft-substratum areas in open waters of western Lake Erie and found infestation of unionids by zebra mussels can equal 100% unionid mortality at individual sites, but not 100% mortality of unionids throughout the study area. However, the presence of living unionids in two nearshore areas in the present study indicate that natural "refugia" could exist in beach/littoral areas of western Lake Erie.

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Effects of quarantine times on glycogen levels of native freshwater mussels (Bivalvia: Unionidae) previously infested with zebra mussels

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Abstract: The effects of zebra mussel infestation and subsequent quarantine on three mussel species were evaluated through glycogen analyses of mantle tissue. Specimens of *Amblema plicata* (Say, 1817) and *Quadrula pustulosa* (I. Lea, 1831), collected from a heavily infested (> 350 zebra mussels/m^2) reach of the Ohio River in 1996, had significantly lower glycogen levels (2.73 and 1.84 mg/g, respectively) than specimens collected from a lightly infested (< 5 zebra mussels/m^2) reach upstream (8.08 and 6.20 mg/g, respectively). Levels of glycogen after 7, 14, and 30 d of quarantine in tanks declined dramatically with length of quarantine. After 30 d without supplemental feeding, mean glycogen levels of *A. plicata* collected from the low density reach had dropped to 15% of that of wild caught specimens (1.22 versus 8.08 mg/g, respectively). After 30 d, mean glycogen levels of *Q. pustulosa* also dropped significantly to 30% of that of wild caught specimens (1.90 versus 6.20 mg/g, respectively). Mean glycogen levels of *Fusconaia ebena* (I. Lea, 1831), collected from the heavily infested reach of the Ohio River dropped to extremely low levels (from 2.75 to 0.53 mg/g) after 30 d of quarantine. Specimens of *F. ebena* were quarantined for an additional 100 d because zebra mussels were found on unionids after 30 and 60 d of quarantine. Feeding every three days between 30-130 d of quarantine was insufficient to allow for recovery after 100 d (0.30 mg/g) or 130 d (0.34 mg/g). A 30 d quarantine of unionids removed from zebra mussel-infested waters causes a significant reduction in glycogen levels which are further reduced if additional quarantine time is required. Feeding of unionids is necessary to maintain their condition during lengthy quarantine, or more effective methods are needed to remove zebra mussels and thus shorten the required quarantine period.

Key words: glycogen, zebra mussels, Unionidae, Ohio River, quarantine

Freshwater mussels of the family Unionidae reach their greatest diversity in North America with nearly 300 species (Williams *et al.*, 1993). However, increased habitat alteration, siltation, and pollution have caused dramatic declines in both species diversity and richness (Bogan, 1993). Populations of freshwater mussels are now at further risk of extirpation or extinction from the exotic zebra mussel, *Dreissena polymorpha* (Pallas, 1771). Since the zebra mussel's introduction into Lake St. Clair around 1985, this exotic mollusk has decimated local populations of freshwater mussels throughout the Great Lakes (Hunter and Bailey, 1992; Gillis and Mackie, 1994; Schloesser and Nalepa, 1994). The pelagic veliger stage has enabled zebra mussels to colonize many of the large river systems of the southeastern United States, and extremely high fecundities have allowed populations to increase exponentially after settlement of veligers (Sprung, 1991). Adult zebra mussels were first collected from the lower Ohio River in 1991 (USACOE, 1993), and densities reached nearly 100,000/m^2 in the lower river by July 1994 (A. Miller, pers. comm.). In 1995, the Ohio River Valley Ecosystem Team identified the potential decline of native aquatic mollusks as its top management priority (USFWS, 1995).

Resource agencies and universities are currently testing removal and translocation as a management tool to conserve declining numbers of unionids from zebra mussel-infested waters. The current protocol in West Virginia for unionid salvage from zebra mussel infested waters requires that all unionids be thoroughly scrubbed to remove zebra mussels (J. Clayton, pers. comm.). Cleaned unionids are then hand-inspected before being placed in aerated quarantine tanks for a minimum of 30 d to allow juvenile zebra mussels missed during the scrubbing procedure to become visible. During quarantine, water quality parameters (*i. e.* temperature, dissolved oxygen, and pH) are monitored to provide suitable conditions for unionid survival. At the end of 30 d, individual unionids are inspected under 10X magnification, and if zebra mussels are found, all specimens must be rescrubbed, placed in clean tanks, and quarantined for an additional 30 d. Finally, translocation can occur only when the native mussels are certified free of zebra mussels.

Zebra mussel infestations in combination with collection, transport, and handling during quarantine, can lead to increased stress in freshwater mussels. Glycogen, an important energy reserve for animals, especially bivalves (de Zwann and Zandee, 1972; Barber and Blake, 1981; Bayne and Newell, 1983; Haag *et al.*, 1993), has been shown to change in response to environmental perturbations such as temperature extremes, anaerobiosis, pollutants, or starvation (de Zwann and Wijsmann, 1976; Hummel *et al.*, 1989). In marine bivalves, glycogen levels also have been shown to change seasonally (Hummel *et al.*, 1988) in response to such factors as gametogenesis (Gabbott, 1983) and winter food shortages (Gade, 1983). Haag *et al.* (1993) showed that the mean glycogen content of *Amblema plicata* (Say, 1817) and *Lampsilis radiata* (Gmelin, 1791) from Lake Erie was significantly lower in zebra mussel-encrusted versus unencrusted control specimens. Thus, a glycogen assay was used in this experiment to assess the impact of zebra mussel infestation, removal, and 30 d quarantine on the physiological condition of freshwater mussels collected from the Ohio River. Specific research objectives were to (1) quantify the glycogen levels of freshwater mussels infested with zebra mussels in high versus low density areas, and (2) assess the change in glycogen levels of unionids during quarantine periods ranging from 30 to 130 d.

METHODOLOGY

The effect of zebra mussel infestation on unionid glycogen levels was compared between specimens collected from high versus low zebra mussel-infested sites on the Ohio River. To minimize the natural, seasonal fluctuation in glycogen levels, specimens were collected from the study sites between 23 July and 21 August 1996. Ten specimens each of *Amblema plicata* and *Quadrula pustulosa* (I. Lea, 1831) were collected from Ohio River Mile (ORM) 175.5 on 23 July 1996. This low infestation site near Parkersburg, West Virginia, had a mean density of 0.3 zebra mussels/m^2, and a maximum of one zebra mussel/unionid (P. Morrison, pers. comm.). On 16 August 1996, ten specimens of *A. plicata* were collected from ORM 967. This heavily infested site near Paducah, Kentucky, had a mean density of 3,600 zebra mussels/m^2 (A. Miller, pers. comm.). Because *Q. pustulosa* was uncommon at ORM 967, ten specimens were collected from ORM 397 on 21 August 1996. Zebra mussel densities at this site near Maysville, Kentucky, increased 30-fold between 1995 and 1996. With a mean density of 360 zebra mussels/m^2 and a maximum of 92 zebra mussels/unionid (P. Morrison, pers. comm.), ORM 397 also was considered to be a heavily infested site. All specimens collected in the field were sacrificed on the day of collection, shucked, weighed, preserved in 95% ethanol, and transported to the laboratory for analysis.

To assess the effect of quarantine on unionid condition, additional specimens of *Amblema plicata* and *Quadrula pustulosa* (250 and 80, respectively) were collected from ORM 175.5. All specimens were aged, measured, tagged, and transported in well water to 300 l, aerated quarantine tanks on Middle Island, Ohio River Islands National Wildlife Refuge, in St. Mary's, West Virginia. Because the quarantine tanks did not provide flow-through conditions, tank water was drained and filled with well water every 2 d. Specimens of *A. plicata* were placed in individual quarantine tanks at densities of 250/m^2 and 65/m^2, respectively, to determine possible density effects on glycogen stores. The 80 specimens of *Q. pustulosa* were placed in a third tank. During the 30 d quarantine, unionids were not fed, simulating likely conditions during recovery, quarantine, and relocation of threatened unionids. Ten specimens of each species were sacrificed from each tank at 7, 14, and 30 d of quarantine, and preserved in 95% ethanol for subsequent glycogen analysis.

Heavily infested individuals of *Amblema plicata* and *Quadrula pustulosa* could not be used to monitor glycogen levels during quarantine because specimens could not be collected in sufficient numbers from the lower Ohio River. Instead, 250 specimens of *Fusconaia ebena* (I. Lea, 1831) were collected from ORM 967 on 16 August 1996, to determine the effect of quarantine on heavily infested unionids. Ten specimens were sacrificed in the field, and the remainder transported to the quarantine site. Again, ten specimens were sacrificed after 7, 14, and 30 d of quarantine, and preserved in 95% ethanol for subsequent glycogen analysis. At the end of 30 d, zebra mussels (3 mm in length) were discovered attached to the umbonal region of five *F. ebena* in quarantine. All specimens were removed, rescrubbed, hand-inspected, and placed in clean quarantine tanks for an additional 30 d. After the initial 30 d, mussels were fed from a fertilized algae tank every 3 d. After 60 d, zebra mussels again were found attached to the umbonal region of five specimens of *F. ebena*, and all specimens were rescrubbed, inspected, and placed in clean quarantine tanks. At the end of 100 d, no zebra mussels were found during inspection but an additional 30 d period was required to assure that no zebra mussels would be transported out of quarantine. After 130 d, unionids were certified free of zebra mussels and removed from quarantine. To assess the effect of this long-term quarantine period, ten specimens were sacrificed after 100 d and 130 d and preserved in 95% ethanol for subsequent glycogen analysis.

The glycogen content of all preserved specimens was determined using the technique described by Keppler and Decker (1974). A 50-100 mg sample of preserved mantle tissue was dissected, blotted dry to remove the ethanol and weighed. Tissue samples were homogenized for 2 hr in 3M perchloric acid and neutralized with 2M KHCO$_3$. Glycogen was converted to glucose with amyloglucosidase (Sigma Chemical Co., St. Louis, Missouri), combined with a dye solution containing o-dianisidine dihydrochloride, and absorbance measured in a spectrophotometer at 450 nm. Total glycogen was determined from a standard curve of glycogen extracted from the blue mussel, *Mytilus edulis* (Linné, 1758), and expressed in milligrams glycogen/gram preserved mantle tissue. It should be noted that 95% ethanol dehydrates tissue and preserved tissue weights likely underestimate wet tissue weights. However, dehydration also reduces error that can result from any change in tissue water levels during stress. Mean glycogen levels were not standardized by total body weight because simple regression revealed no correlation between wet weight and glycogen content ($r^2 < 0.10$). The mean glycogen levels of all treatments (high versus low zebra mussel density and 7-30 d of quarantine) were normally distributed according to the Kolmogorov-Smirnov goodness of fit test ($\alpha = 0.05$). However, zebra mussel infestation and starvation during quarantine uniformly decreased the glycogen levels of all specimens and consequently decreased overall variance. Following Lentner (1993), the sample variances were equalized using the square root of each individual glycogen value. Converted mean glycogen levels were then compared using ANOVA. If significant differences were detected, Scheffe F-test was used to determine the statistical significance of individual treatments.

RESULTS

Initial mean glycogen levels of *Amblema plicata* collected from the heavily infested site (ORM 967) were significantly lower ($p < 0.05$) than those collected from the upper river at ORM 175.5 (2.73 ± 2.81 mg/g versus 8.08 ± 4.26 mg/g, respectively). The initial mean glycogen level of *Quadrula pustulosa* collected from ORM 397 also was significantly lower ($p < 0.05$) than that collected from ORM 175.5 (1.84 ± 1.23 mg/g versus 6.20 ± 2.89 mg/g, respectively). During quarantine, the mean glycogen level of *A. plicata* collected from ORM 175.5 dropped significantly ($p < 0.05$) after 7 d (Fig. 1). While significant differences were not observed between 7 and 14 d ($p > 0.3$), the mean glycogen level continued to drop significantly ($p < 0.05$) between 14 and 30 d until reaching 15% of that measured in wild-caught specimens (Fig. 1). The mean glycogen level of *Q. pustulosa* collected from ORM 175.5 also dropped significantly ($p < 0.05$) after 7 d of quarantine (Fig. 1). Between 7 and 14 d, the mean glycogen level increased; however, the increase was not statistically significant ($p > 0.1$). At 30 d, the mean glycogen level dropped significantly ($p < 0.05$) to only 31% of that measured in wild-caught specimens (Fig. 1). There was no significant difference ($p > 0.3$) in mean glycogen levels between mussels held at 250/m^2 and 65/m^2 after 7 d (3.56 ± 1.78 mg/g and 4.09 ± 2.18 mg/g, respectively) or 14 d (3.27 ± 1.74 mg/g and 3.10 ± 1.57 mg/g, respectively).

Specimens of *Fusconaia ebena* collected from ORM 967 also showed a significant decline ($p < 0.05$) in the mean glycogen level after 7 d of quarantine (Fig. 2). However, significant changes were not detected for the remainder of the quarantine period. After 30 d, the mean glycogen level was only 20% of that measured in wild-caught specimens (Fig. 2). Feeding of unionids every 3 d between 30 and 130 d was not sufficient to allow unionid glycogen levels to recover. After 130 d, the mean glycogen level was only 12% of that measured in wild-caught specimens (Fig. 2).

DISCUSSION

While different densities (up to 250 unionids/m^2) in quarantine had no significant effect on the glycogen stores of *Amblema plicata*, it is clear that previous levels of zebra mussel infestation and starvation during quarantine significantly reduce unionid energy stores. By attaching in great densities to the outer shell of living unionids, zebra mussels reduce glycogen stores, presumably by reducing vital food resources, disrupting proper feeding and respiration, and preventing valve opening and closing (Mackie, 1991).

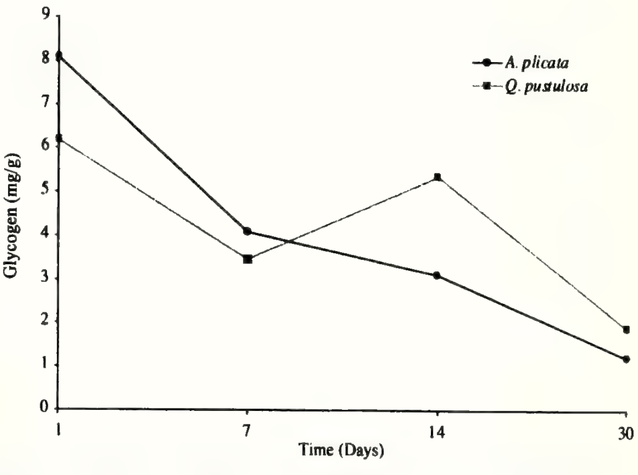

Fig. 1. Glycogen levels (mg/g) of *Amblema plicata* and *Quadrula pustulosa* at 1, 7, 14, and 30 d of quarantine (n = 10/sampling period).

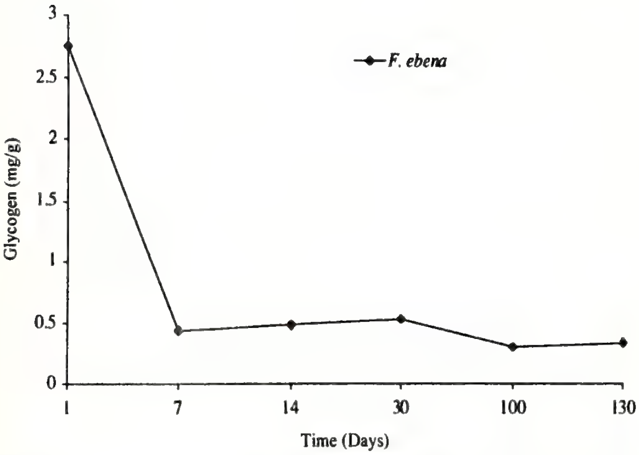

Fig. 2. Glycogen levels (mg/g) of *Fusconaia ebena* at 1, 7, 14, 30, 100, and 130 d of quarantine (n = 10/sampling period).

Thus, energy stores were already at low levels when unionids entered the 30 d quarantine period. Assay results from *Fusconaia ebena* revealed that low glycogen levels of unionids removed from areas with high densities of zebra mussels reach dangerously low levels during a 30 d quarantine period. It is unclear whether a threshold level of glycogen is required to cause mortality, but low energy stores after quarantine can decrease the likelihood that unionids will survive the relocation process. Unionids collected from high quality habitat with low zebra mussel densities can have sufficient energy stores to survive a quarantine period and subsequent translocation, however, unionids in areas with high densities of zebra mussels are the primary candidates for relocation. Thus, when unionids from zebra mussel-infested waters are translocated, a major limiting factor could be the physiological condition and energy reserves of unionids at the time of relocation.

In a review of the literature, Cope and Waller (1995) reported that survival of translocated unionids is typically low (< 50%) and is influenced by many factors. Factors affecting translocation success such as habitat suitability, numbers of individuals released, and the frequency of release have been given significant attention in recent years for both terrestrial and aquatic organisms (Griffith *et al.*, 1989; Cope and Waller, 1995). However, no attention has been given to the physiological condition or energy reserves of relocated organisms. In order to reduce the likelihood of latent mortality of mussels salvaged from zebra mussel-infested waters, it is necessary to either provide sufficient food and favorable water quality conditions during quarantine or to have a brief quarantine period to ensure that unionids have sufficient energy stores to recover from the stressful relocation to new environments.

As judged by the energy reserves in specimens of *Fusconaia ebena* from 30-130 d of quarantine, starved unionids can reach a point where supplemental feeding contributes little to the recovery of energy reserves. Thus, a detailed study to determine the amount of food required to maintain unionid condition during quarantine is needed. In addition to maintaining unionid condition, food supplements also will increase the growth rate of juvenile zebra mussels that are missed during the scrubbing procedure. It is evident that small zebra mussels can avoid extensive scrubbing and inspection, possibly by residing in the crevices of damaged shells. Because the purpose of quarantine is to guarantee the absence of zebra mussels, increased growth rates would enhance detection and justify a reduction in the quarantine period. More effective techniques of zebra mussel removal also should be developed to reduce or perhaps eliminate the need for a lengthy quarantine period.

Assay results from this study reveal that the glycogen levels of all three species decreased significantly after 7 d, and then stabilized between 7 and 14 d. Thus, a reduction in the quarantine period from 30 to 15 d would greatly improve the overall condition of unionids prior to translocation. However, under current protocol standards, unionids must endure a minimum of 30 d of quarantine and a total of 60 d if zebra mussels are detected, which can cause glycogen levels of unionids to decline to life-threatening levels. Thus, one of the greatest concerns during the salvage of zebra mussel-infested unionids should be the physiological condition of unionids at the time of their final relocation.

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Research Note

Observations on the reproductive biology of the octopod *Eledone gaucha* Haimovici, 1988, in southern Brazil

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Abstract: Maturation, fecundity and reproductive cycle of the octopod *Eledone gaucha* Haimovici, 1988, were studied based on preserved samples collected during groundfish surveys conducted off southern Brazil. The patterns of sexual maturation of males and females and low fecundity were similar to the congeneric and sympatric *E. massyae* Voss, 1964. It is suggested that *E. gaucha* does not exhibit a marked seasonal reproductive cycle and more than one breeding group overlap on the continental shelf.

Key words: Octopoda, *Eledone*, sexual maturation, Brazil

The small congeneric octopods, *Eledone massyae* Voss, 1964, and *E. gaucha* Haimovici, 1988, coexist year-round on the sandy and muddy bottoms of the middle and outer continental shelf off southern Brazil (Haimovici and Andriguetto, 1986; Haimovici and Perez, 1991a, b). A series of groundfish surveys conducted between 1982 and 1987 by the R/V "Atlântico Sul" of the Rio Grande University (Table 1), collected both species between 33°45' and 30°43' S and the isobaths of 40 and 160 m. Specimens of *Eledone* were fresh-frozen or preserved on board, for comparative genetic and morphological studies (Haimovici, 1988; Levy *et al.*, 1988) and basic descriptions of their population structure, reproductive biology, and ecology (Perez *et al.*, 1990; Perez and Haimovici, 1991, 1995). A more detailed study on maturation and reproductive cycle was conducted on the larger and more commonly trawled *E. massyae* (*fide* Perez and Haimovici, 1991). Less information was available on the smaller and scarcer *E. gaucha*. Our observations on the reproductive biology of this species are summarized in this note.

A total of 88 males and 95 females of *Eledone gaucha* (Table 1) were fixed in 10% formalin and preserved in 70% ethanol. All specimens were weighed, measured (ML, dorsal mantle length) in millimeters, and their gonads and gonoducts dissected out and weighed to the nearest 0.1 g. Macroscopic maturity stages were assigned to males and females according to the scale proposed for *E. massyae* (*fide* Perez and Haimovici, 1991). For females the stages were: I, immature; II, early maturation; III, maturing; IV, advanced maturity. The female maturity scale does not consider fully mature or spawned ovaries, because these were not found in the samples. For males the stages were: I, immature; II, maturing; III, mature (with functional spermatophores in the spermatophore sac); IV, spent. In females, all eggs were counted and length measured to the nearest 0.1 mm and the presence of sperm sacs (evaginated spermatophores) and free sperm within the ovary were recorded as evidence of mating. In males, all spermatophores stored in the spermatophore sac were counted.

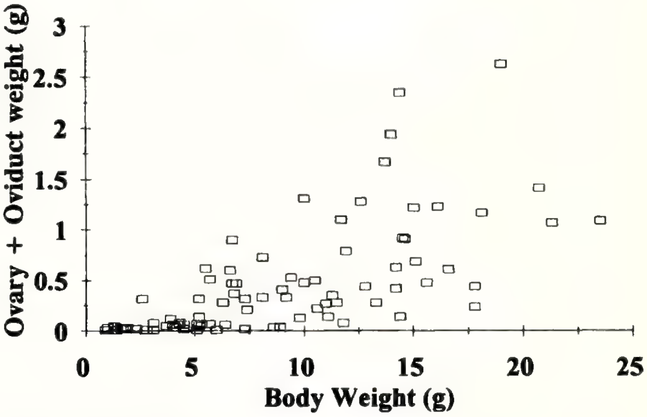

Fig. 1. Ovary and oviduct weight as a function of total body weight of female *Eledone gaucha* collected off southern Brazil.

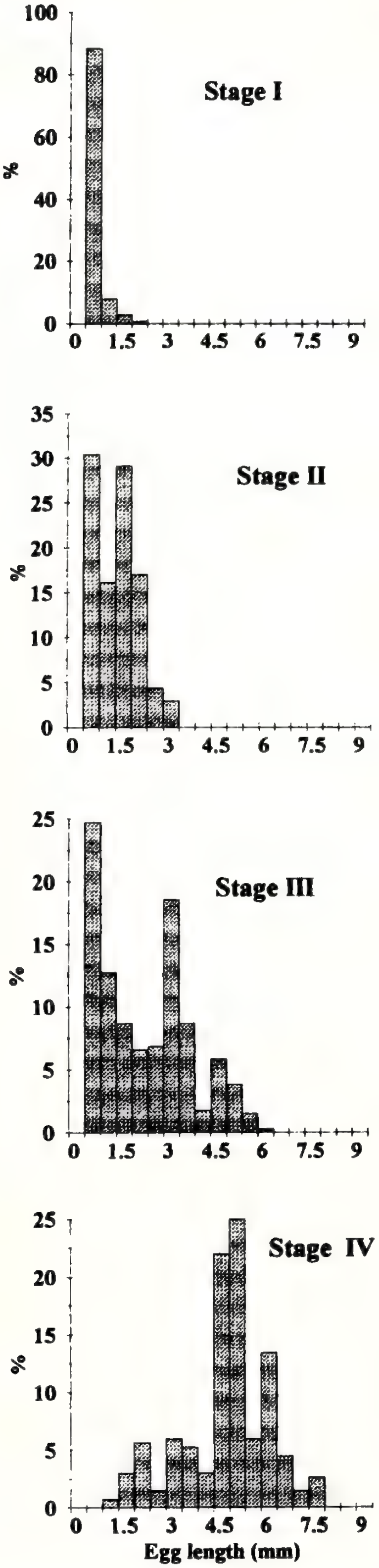

Fig. 2. Length-frequency distribution of ovarian eggs of female *Eledone gaucha* pooled by maturity stages: I, immature; II, early maturation; III, maturing; IV, advanced maturity.

Females ranged from 14 to 55 mm ML and 0.9 to 25.5 g wet weight (Table 1). The ovary and oviducts showed enlargement in females heavier than 5.0 g and reached a maximum of 17% of total weight, varying greatly at a given body size (Fig. 1). In the examined ovaries, most of the eggs enlarged at about the same time throughout maturation (Fig. 2). A bimodal distribution was observed in females at stage III. In the most advanced stage IV, most eggs concentrated around a single mode (5.0 mm). The largest eggs were *ca.* 7.4 mm long as observed in a female (14.4 g wet weight) caught in June 1980. Even these eggs, however, were longitudinally striated indicating that they were not fully mature. Neither mature nor spent females were caught during this study. Mated females, with free sperm and sperm sacks in the ovary, were caught in all sampled months mostly in females in advanced maturity stages. The smallest maturing female with evidence of mating was 16.6 mm ML with mean egg length of 0.84 mm.

The number of eggs present in the ovary ranged from 5 to 58 (n = 91, mean 35 ± 12.7 SD), and the relative fecundity (eggs per gram of total weight) ranged from 0.68 to 51.11 (n = 91, mean 7.4 ± 8.7 SD). No significant linear correlation was found between the number of eggs and female weight ($r = 0.031$; $p > 0.50$) or mantle length ($r = 0.035$; $p > 0.50$).

Males ranged from 14 to 47 mm ML and 1.2 to 17.8 g wet weight (Table 1). Genital bag (testis, spermatophore sac, and spermatophore glandular systems) enlargement had begun in males heavier than 3.0 g and larger than 22.0 mm ML, and reached a maximum of 9.5% of the total weight (Fig. 3). Spermatophores were observed in males as small as 20 mm ML. Half of the males carrying spermatophores in the spermatophore sac, however, were larger than 31 mm ML. A significant positive correlation

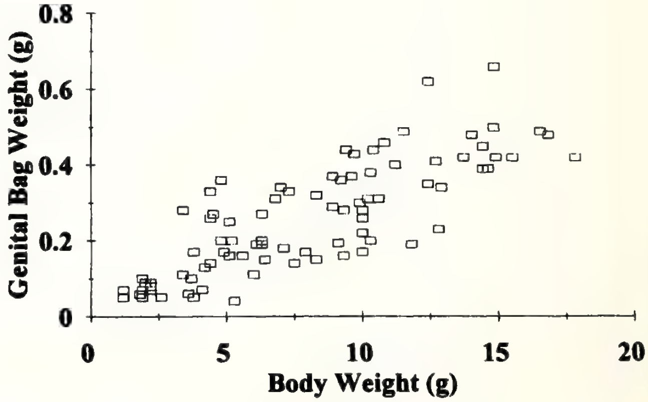

Fig. 3. Genital bag weight (testis, spermatophore glands, and spermatophore sac) as a function of total body weight of male *Eledone gaucha* collected off southern Brazil.

Table 1. Summary of *Eledone gaucha* samples collected in 11 surveys conducted from 1980 and 1985 off southern Brazil. n, sample size; ML, mantle length.

			Males			Females		
Year	Month	Depth (m)	n	ML (mm) min - max	Weight (g) min-max	n	ML (mm) min-max	Weight (g) min-max
1980	June	11-140	2	26	5.2 - 6.1	6	32 - 55	3.1 - 19.0
1980	July	40-120	2	30 - 34	8.9 - 14.8	0		
1981	April	13-89	6	24 -34	6.3 - 10.0	0		
1982	January	10-63	20	14 - 44	1.2 - 13.7	20	14 - 35	0.9 - 10.0
1983	April	13-122	17	18 - 34	2.6 - 10.0	17	15 - 41	1.4 - 14.4
1983	July	—	1	44	13.7	0		
1983	August	14-110	21	26 - 45	3.4 - 14.8	23	25 - 44	1.9 - 25.5
1983	November	10-100	4	17 - 47	1.2 - 17.8	7	31 - 47	1.9 - 18.0
1984	January	12-200	2	24 - 35	6.0 - 11.8	4	30 - 35	10.5 - 15.0
1984	November	10-197	8	20 - 43	2.2 - 16.8	7	41 - 49	14.2 - 25.5
1985	September	—	1	37	12.9	11	25 - 34	5.2 - 12.8
Totals			88	14 - 47	1.2 - 17.8	95	14 - 55	0.9 - 25.5

was found between the number of spermatophores stored in the spermatophore sac and male weight ($r = 0.522$; $p < 0.001$), mantle length ($r = 0.303$; $p < 0.005$) and genital bag weight ($r = 0.446$; $p < 0.001$). The maximum number of spermatophores observed in a single male was 82 (mean 23.9 ± 3.6 SD, n = 86). Males with spermatophores occurred in all sampled months.

The maturation cycle was assessed by the variation of the gonad index (gonad weight expressed as a proportion of the total wet weight) of individuals collected during all surveys pooled by month (Figs. 4 and 5). Octopods in both initial and advanced stages of maturation co-occurred in all sampled months. The pattern was less clear among males because young individuals were probably not well represented in the samples (Perez and Haimovici, 1995). Whereas the data suggest an overlap of generations within a year, the duration of the maturation cycle could not be defined. The pattern observed, however, could be an artifact of pooling individuals collected in different years by month, specially if the reproductive cycle is shorter than one year, such as that of other small octopodids (Forsythe, 1984). Because the data set available is scarce and sparsely distributed within the five-year period of sampling, an adequate test of the latter hypothesis was not possible.

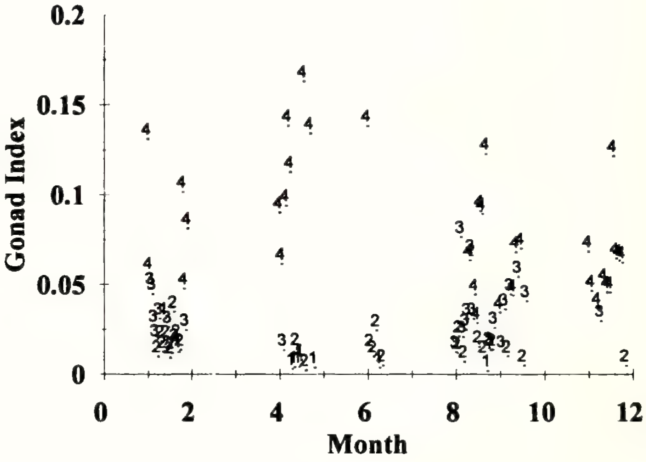

Fig. 4. Monthly variation of gonad indices of female *Eledone gaucha* collected off southern Brazil. The numbers represent maturity stages assigned for each individual: 1, immature; 2, early maturation; 3, maturing; 4, advanced maturity.

The maturation processes of *Eledone gaucha* are consistent with those of *E. massyae* (*fide* Perez and Haimovici, 1991) and in the European species, *E. cirrhosa* (Lamarck, 1798) and *E. moschata* (Lamarck, 1798) (Mangold, 1983; Boyle and Knobloch, 1983; Moriyasu, 1988). Females reach maturity later and at a wider range of sizes than males. Females can mate while still not fully mature and store sperm in the apical filaments of the eggs until vitellogenesis is completed (Perez *et al.*, 1990). Mature and spent females were not vulnerable to the trawl net. As pointed out in previous studies (Perez and Haimovici, 1991, 1995), this could relate to migration offshore, out of the study area, towards deep rocky bottoms suitable for spawning. Although few animals were present in each sample of *E. gaucha*, there was no evidence of seasonality in the reproductive cycle in contrast to that of *E. massyae*, in which mating and spawning were concentrated in the spring and autumn, respectively (Perez and Haimovici, 1991). In addition, as discussed in a previous study (Perez and Haimovici, 1995), more than one cohort of *E. gaucha* seem to overlap throughout the year, each of them with reproductive cycles of uncertain duration but possibly sub-annual. If confirmed, this feature plus dietary differences during the adult phase (Perez and Haimovici,

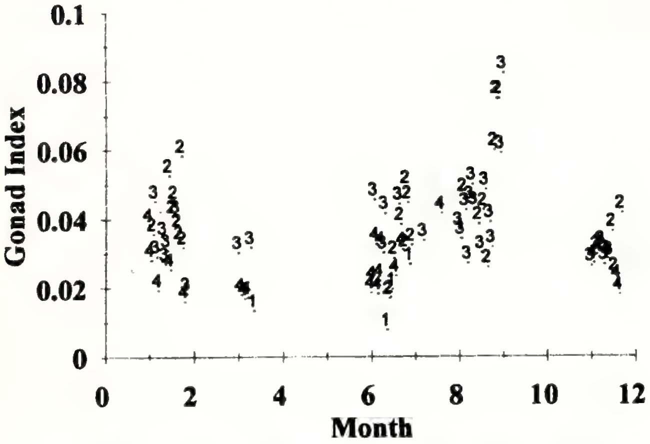

Fig. 5. Monthly variation of gonad indices of male *Eledone gaucha* collected off southern Brazil. The numbers represent maturity stages assigned for each individual: 1, immature; 2, early maturation; 3, mature; 4, spent.

1995) could allow ecological niche divergence between these two sympatric octopods.

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64th ANNUAL MEETING
THE AMERICAN MALACOLOGICAL UNION
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In 1998 the American Malacological Union will meet jointly with Unitas Malacologica and the Western Society of Malacology in Washington, D. C. The meeting site is the Smithsonian Institution on the National Mall. A large number of malacologists from outside of North America are expected to attend, and you are urged to participate in what surely will be a unique meeting. A detailed meeting announcement, registration, materials, and the call for papers and posters can be downloaded from the AMU web site (http://erato.acnatsci.org/amu).

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   Organizers: Tim Collins (collins@servms.fiu.edu), Gerhard Haszprunar, Diana Lipscomb, and Winston Ponder.

2) Interactions between Molluscs and Humans
   Organizers: Philippe Bouchet, George Davis (davis@say.acnatsci.org), Eric Hochberg, and Gerrado Vasta

3) Bridging Temporal Scales in Malacology: Uniting the Living and the Dead
   Organizers: Satoshi Chiba, Douglas Erwin (erwin.doug@nmnh.si.edu) and David Reid

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Three social events are planned, highlighted by a concluding evening cruise along the Potomac River. Please note that the single registration fee includes all of the social events! During open evenings participants will be encouraged to take advantage of the fine dining opportunities in the D. C. area. Washington is a veritable tourist mecca and participants also should plan on spending time visiting some of the museums, galleries, monuments, federal buildings,and parks.

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Boucardicus victorhernandezi, a new, endangered species of cyclophorid land snail from Madagascar

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Abstract: *Boucardicus victorhernandezi* sp. nov. is known from only four mountains in the Vohimena chain, which stretches for 75 km between the Indian Ocean coast and the Anosy chain, north of Tolagnaro (Fort Dauphin), Madagascar. Vohimena-chain forests, of which less than 500 km^2 remain, are effectively unprotected and are being deforested rapidly.

Key words: Gastropoda, Cyclophoridae, tropical rainforest

This paper describes a new, recently discovered species of land snail from primary rainforest in southeastern Madagascar. This species is known only from the Vohimena mountain chain, north of Tolagnaro (Fort Dauphin). The remnant forests of the Vohimena chain are now under 500 km^2 in extent, are under no special protection, and are undergoing rapid deforestation (Emberton, 1997; Emberton *et al.*, in press). In the current Red List of Threatened Animals (IUCN, 1996), Madagascar currently has four land-snail species listed: two Endangered and two Vulnerable. The new species described herein is proposed for listing as Endangered.

The cyclophorid genus *Boucardicus* Fischer-Piette and Bedoucha, 1965, is known only from Madagascar, where it ranges throughout much of the island (Fischer-Piette *et al.*, 1993; Emberton, 1995, in press). Morphologically, the *Boucardicus* shell varies from globose to high-spired, from about 1-8 mm in greatest dimension, from dentate to edentate, from double- to single-lipped, from unlobed to trilobed lip, etc., with complex variations in body-whorl constriction, post-constriction ribbing, apertural configuration, microsculpture, etc. The new species described herein expands the known variation in shell form to include a bilobed apertural lip.

Ecologically, *Boucardicus* seems to represent the most diverse land-snail radiation of Madagascar's rainforests, as well as their strongest indicator of ecological degradation (Emberton, 1997, in press; Pearce and Emberton, unpub.). For conservation biology, *Boucardicus* qualifies well as an indicator/target taxon (di Castri *et al.*, 1992; Kremen, 1994), because it is an endemic, species-rich, well-defined clade, with species readily identifiable by shells alone (Emberton, 1996). For biogeography, *Boucardicus* ranks among the most informative of Madagascar's land-snail taxa (Emberton and Rakotomalala, 1996). This new species increases the number of described *Boucardicus* species from 39 (Fischer-Piette *et al.*, 1993; Emberton, 1994) to 40.

METHODS AND MATERIALS

Drawings by camera lucida and measurements by ocular micrometer are all by the author, using a Wild M5A dissecting microscope. Some measurements were taken from photographs of the holotype to be published separately in a systematic treatment of Vohimena chain-Anosy chain *Boucardicus* (Emberton and Pearce, in press).

The description was prepared in part using DELTA (Dallwitz *et al.*, 1993). The description is conchological only.

Endangered-species status was evaluated using the latest categories and criteria of the International Union for the Conservation of Nature (IUCN, 1996). Rainforest extent and decline were assessed using Green and Sussman (1990), Sussman *et al.* (1994), and the most recently available topographic maps.

SYSTEMATICS

Higher classification follows Vaught (1989) and

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Ponder and Lindberg (1997). Types are placed in the National Museum of Natural History, Washington, D. C. (USNM); the Muséum national d'Histoire naturelle, Paris (MNHN, which does not assign catalog numbers to its types); the Australian Museum, Sydney (AMS); and, temporarily, the Molluscan Biodiversity Institute (MBI). All MBI collections are destined in the near future for USNM (MBI 373-379) and the Academy of Natural Sciences of Philadelphia (ANSP) (MBI 1419-1445).

Class GASTROPODA
Clade CAENOGASTROPODA
Superfamily CYCLOPHOROIDEA
Family CYCLOPHORIDAE
Genus *Boucardicus* Fischer-Piette and Bedoucha, 1965

Boucardicus victorhernandezi sp. nov.

Figs. 1-2

Boucardicus sp. 5. -Emberton *et al.*, 1996: 210; Emberton, 1997: 1147; Emberton *et al.*, in press: table 2.

HOLOTYPE. USNM 860775 (ex MBI 381.02DH, 1 adult shell, illustrated): 24°51'39"S, 47°00'46"E, Madagascar, Tulear Province, N of Fort Dauphin, W of village of Mahialambo, Mount Ilapiry, ESE slope, 200 m, primary rainforest, 02 Feb 1995.

PARATYPES. MNHN (1 adult shell, ex MBI 1447.01DP): 24°26'15"S, 47°13'10"E, Madagascar, Tulear Province, N of Fort Dauphin, NE of village of Esetra, Mount Mahermana, W slope, ca. 250 m, primary rainforest, 15 Oct 1992. AMS C.203423 (1 adult shell, ex MBI 380.08DP): 24°51'36"S, 47°00'40"E, Madagascar, Tulear Province, N of Fort Dauphin, W of village of Mahialambo, Mount Ilapiry, S slope, 300 m, primary rainforest, 02 Feb 1995. MBI 373.23AP, destined for USNM (1 adult female, alcohol preserved): 24°26'12"S, 47°13'13"E, Madagascar, Tulear Province, N of Fort Dauphin, NE of village of Esetra, Mount Mahermana, summit, 340 m, primary rainforest, 25 Jan 1995. MBI 376.20AP, destined for USNM (1 adult female, alcohol preserved): 24°26'22"S, 47°12'41"E, Madagascar, Tulear Province, N of Fort Dauphin, NE of village of Esetra, Mount Mahermana, valley in NW slope, 100 m, primary rainforest, 27 Jan 1995. MBI 377.07DP, destined for USNM (1 adult shell): 24°51'40"S, 47°00'20"E, Madagascar, Tulear Province, N of Fort Dauphin, W of village of Mahialambo, Mount Ilapiry, summit, 540 m, primary rainforest, 30 Jan 1995. MBI 379.33AP, destined for USNM (1 adult female, alcohol preserved): 24°51'27"S, 47°00'38"E, Madagascar, Tulear Province, N of Fort Dauphin, W of Mahialambo, Mount Ilapiry, SE slope, 400 m, primary rainforest, 31 Jan 1995. MBI 1419.01DPR, destined for ANSP (1 adult shell, illustrated): 25°00'30"S, 46°57'45"E, Madagascar, Tulear Province, NW of Fort Dauphin, Pic Saint Louis, near summit, ca. 500 m, small remnant patch of primary rainforest, 10 Oct 1992. MBI 1439.01AP, destined for ANSP (7 adults and 2 juveniles, plus 1 adult body, alcohol preserved); MBI 1439.01DP (1 dead juvenile); MBI 1439.01DPR (1 adult shell, illustrated; 1 juvenile shell): 24°22'05"S, 47°08'00"E, Madagascar, Tulear Province, N of Fort Dauphin, S of village of Esetra, Mount Vohibololo (local name), summit, 430 m, primary rainforest, 14 Oct 1992. MBI 1445.01AP, destined for ANSP (1 adult, alcohol preserved): 24°26'12"S, 47°13'13"E, Madagascar, Tulear Province, N of Fort Dauphin, NE of village of Esetra, Mount Mahermana, summit, 340 m, primary rainforest, 15 Oct 1992.

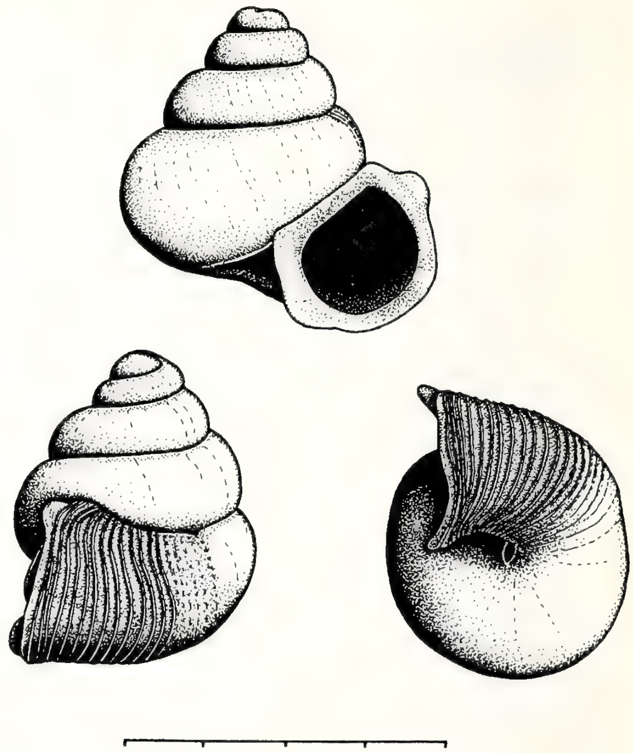

Fig. 1. Holotype, *Boucardicus victorhernandezi*, from Mt. Ilapiry (USNM 860775). Scale bar = 4 mm.

TYPE LOCALITY. Madagascar, Tulear Province, N of Fort Dauphin, W of village of Mahialambo, Mount Ilapiry, ESE slope, 200 m, 24°51'39"S, 47°00'46"E.

DIAGNOSIS. A *Boucardicus* with bilobed apertural lip and strongly ribbed preapertural sculpture.

DESCRIPTION OF HOLOTYPE. Shell size and shape.

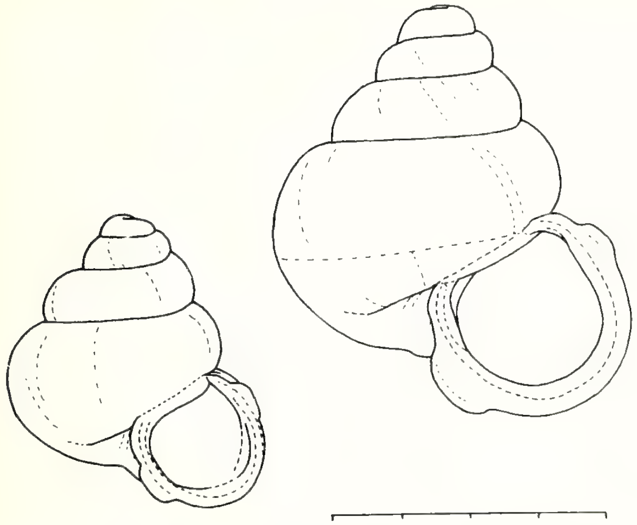

Fig. 2. Paratypes, *Boucardicus victorhernandezi,* from Pic St. Louis (left, MBI 1419.01DPR) and Mt. Vohibololo (right, MBI 1439.01DPR). Scale bar = 4 mm.

Diameter 3.7 mm; height 3.8 mm. Height-diameter ratio 1.0. Spire angle 80°. Whorl periphery round. Whorl shoulder round. Umbilicus before body whorl constriction 0% of shell diameter. Final umbilicus 29% of shell diameter. Whorls 4.5.

Aperture. Aperture width parallel to parietal callus 38% of shell diameter. Aperture height-width ratio (height measured perpendicular to parietal callus) 0.87. Columellar plica absent. Apertural plane inclined upward; 5° from rotational axis. No apertural anal notch. No baso-columellar denticle. No basal denticle. No upper palatal denticle. Ratio of aperture plus peristome greatest width (measured parallel to or within 40° of parietal-callus line) to aperture width 1.54. Aperture plus peristome greatest dimension angled outward from rotational axis 35°. Ratio of aperture plus peristome greatest width to aperture plus peristome greatest height as measured perpendicular to width line 1.32. Peristome baso-palatal indentation 23% of basal peristome width. Peristome upper curl extending forward 17% of upper peristome width. Inner, second peristome present, projecting less than 0.01 whorl.

Apex. Embryonic whorls 2.0. Embryonic sculpture granular with faint traces of growth lines. First whorl diameter 0.75 mm. First three whorls diameter 2.10 mm.

Sculpture on last tenth of penultimate whorl. Transverse ribs 22; rib height less than 0.05% of shell diameter. No complete spiral grooves between sutures. No short spiral grooves between sutures. No spiral ridges between sutures. No spiral lines of punctae between sutures. No herringbone sculpture; no honeycomb sculpture.

Pre-Apertural Morphology. Body whorl constricted 0.3 whorl before aperture; constricting by 11% of whorl diameter. Body whorl sculpture not diminishing before constriction. Post-constriction body whorl swollen by 10% of constriction diameter. No secondary body-whorl constriction. Transverse ribs on post-constrictional swelling numbering 10 in 0.1 whorl; rib height 1.1% of shell diameter; ribs slanting forward 80°.

Color. Basic color brown-red. Apex dark brown-red. One spiral color band; color white. Pre-apertural constriction white. Peristome (excluding periostracum) white and red-brown.

VARIATION. Shell size varies considerably, up to 6.0 mm in height (Fig. 2). Shape variation is minimal (Figs. 1-2).

COMPARISONS. Unique among all known *Boucardicus* in its bilobed apertural lip and in the strongly ribbed sculpture of its preapertural body whorl.

DISTRIBUTION. Known only from the Vohimena mountain chain, southeastern Madagascar.

ETYMOLOGY. For Victor Lee Hernandez, amateur naturalist and invertebrate enthusiast, of Commerce, California. On the occasion of his 30th birthday, Christmas Day, 1997, Victor's friends sponsor this species on his behalf.

ENDANGERED STATUS

Evaluation must rely on IUCN's (1996) Criterion B: Small Distribution and Decline or Fluctuation. Use of other IUCN criteria is not possible, because population levels and rates of decline are unknown for this species (or for any other Madagascan land-snail species).

Madagascar has been sufficiently surveyed now for land snails (Emberton, in press), so that the range of *Boucardicus victorhernandezi* can be delimited to the Vohimena mountain chain. Extensive collections north (closest: mountains near Midongy Atsimo and eastward), east (coastal forests, including Sainte Luce), west (several transects in the Anosy chain, including two in Andohahela National Park, and one transect through the mountain ridge joining the Anosies and the Vohimenas), and south (coastal forests) of the Vohimenas, all failed to turn up a single specimen of *B. victorhernandezi.*

Boucardicus victorhernandezi is known from only four mountains within the Vohimena chain: Pic Saint Louis and Mounts Vohibololo, Mahermana, and Ilapiry. Extensive collections from five other mountains in the chain - Pic Saint Jacques (24°58'S, 46°58'E) and Mounts Varabe (24°49'S, 47°04'E), Teloboko (24°28'S, 47°11'E), Rianabo (24°29'S, 47°11'E), and Mahiamalo (24°26'S, 47°11'E) - were devoid of this species. On two of the four mountains from which it is known, *B. victorhernandezi* is known from just a single locality at or near the summit: Pic Saint Louis and Mount Vohibololo.

The Vohimena mountain chain starts at about latitude 24°25'S and stretches south-southeast, parallel to the nearby Indian Ocean coastline, to about latitude 25°01'S. Its length is approximately 75 km. The Vohimena chain's remaining rainforest coverage in 1950 (date of the aerial photos upon which current topographic maps are based) occurred along its entire length and varied in width from 4-16 km, averaging about 10 km. Thus the 1950 area of Vohimena-chain rainforests was approximately 750 km^2.

By 1985, these rainforests had been reduced by about a third, to approximately 500 km^2, judging from satellite-imagery interpretations by Green and Sussman (1990: fig. 1) and Sussman *et al.* (1994: fig. 1). Vohimena-chain rainforests continue to decline at an ever accelerating rate because of timber concessions and slash-and-burn agriculture (Emberton, 1997, pers. obs.; M. Fenn, World Wildlife Fund, pers. comm.). Current plans by an international mining company to begin exploiting nearby titanium resources will dramatically increase the already rapidly growing local human population, leading to even greater rates of forest-clearing (M. Fenn, pers. comm.).

Thus *Boucardicus victorhernandezi* meets strictest IUCN (1996) criteria for Endangered status. Its area of occupancy (Criterion B) is less than 500 km^2. Its distribution is severely fragmented, and it is known to exist at fewer than five locations (Criterion B.1). Its extent of habitat is continuing to decline (Criterion B.2). *B. victorhernandezi* is clearly an Endangered species.

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Differences in the ecology and distribution of lotic pulmonate and prosobranch gastropods

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Abstract: We hypothesize that pulmonate gastropods, in comparison to prosobranchs should (1) be better competitors because of finely-toothed radulae, (2) be better adapted to variable habitats because of greater powers of physiological regulation, (3) be better dispersers, but (4) face greater risk of predation. In the Salt River, in western Kentucky, U. S. A., pulmonates were fairly common in headwater streams, and were replaced in larger order tributaries by prosobranch snails. The literature suggests that pulmonates have tolerance and capacity adaptations to variation in physicochemical variables like temperature or dissolved oxygen. Only one of the two pulmonates tested in the laboratory, however, had quicker righting responses and rates of movement, important in recolonizing ephemeral habitats. A competition experiment in an artificial stream indicated that the large-river prosobranch, *Lithasia obovata* (Say, 1829), suffered less reduction in growth than the pulmonate *Helisoma trivolvis* (Say, 1817). Both the literature and experiments we have performed suggest that prosobranchs are less vulnerable to predators. We interpret these results to suggest pulmonates are more common in temporary headwater streams, or shallow littoral margins of lotic systems, because of greater physiological adaptation, while prosobranchs are more common in larger rivers (or in spring-fed rivers) because of greater competitive ability, lower risk of predation, or less-variable physicochemical regimes.

Key words: pulmonates, prosobranchs, distributions, competition, predation

The threat of extinction to freshwater unionid mussels from habitat alteration and the introduction of exotic species is well known, with almost one-third of the approximately 300 species in the United States either endangered or threatened (Neves *et al.*, 1998). In comparison, freshwater gastropods are even more diverse, with over 500 species recorded in the United States, and an especially high diversity in the southeastern states. Particularly at risk are the diverse Mobile and Tennessee River basin hydrobiids and pleurocerids, whose ecology and life histories are relatively unknown (Neves *et al.*, 1998). It is likely that this rich gastropod fauna is susceptible to some of the same threats as are unionids: impoundments, exotic species, channelization, and pollution (Jenkinson and Todd, 1998).

Although they have not received the attention that unionids have garnered, lotic gastropods can be important to ecosystem function. High densities of gastropod grazers can result in shifts in periphyton assemblages from long filamentous green algae, to more resistant forms such as adnate diatoms or toxic cyanobacteria (reviewed by Brown, 1991). For example, the pleurocerid *Elimia* can reduce periphyton biomass in southeastern rivers (see Rosemond *et al.*, 1993, and others reviewed therein). Because lotic gastropods are so diverse, are potentially at risk, and have important impacts on riverine ecosystems, more emphasis should be placed on understanding their ecology. The objective of this paper is to take a step in this direction by trying to understand ecological interactions between two prominent gastropod groups found in lotic systems, *i. e.* pulmonate and prosobranch snails.

Our approach is to review the ecology and basic biology of both groups, and formulate some hypotheses for why their habitat distributions differ. We argue that key factors explaining these distributional differences include relative dispersal abilities, competitive abilities, physiological adaptations to stressor variables, and predation risk.

Comparative ecology of pulmonates and prosobranchs

Pulmonates and prosobranchs differ in their evolutionary biology, ecology, and impact on lotic systems (Russell-Hunter, 1978; Brown, 1983, 1991; McMahon, 1983). Pulmonates have secondarily reinvaded freshwaters (from terrestrial intermediates) and range from amphibious to fully aquatic. Members of the families Lymnaeidae and Physidae rely on aerial respiration and are somewhat amphibious, whereas ancylids (limpets) and planorbids have secondarily-derived epithelial gills or dedicated respiratory pigments and are truly aquatic. The gilled prosobranchs evidently invaded lotic systems from estuaries

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(McMahon, 1983) and many have longer life cycles, reduced reproductive effort, and greater iteroparity in comparison to most pulmonates (Browne, 1978; Browne and Russell-Hunter, 1978; Aldridge, 1983; Brown, 1983). Prosobranchs are often more streamlined (with the exception of the pulmonate ancylids) and therefore more resistant to being displaced by currents (Dussart, 1987). For example, pulmonate planospiral or globose shell shapes (*Helisoma* [= *Planorbella*] or *Physella* [= *Physa*], respectively) have higher drag coefficients. In fact, the only prosobranch family with globose shells, the viviparids, are characteristic of lentic or lotic habitats with little flow. Pulmonates, because of their "lungs," can passively disperse on birds (see references in Brown, 1991), and they therefore could have higher dispersal rates and lower levels of endemism (Davis, 1982).

Consequently, we could expect that pulmonates, with their higher reproductive rates, shorter life cycles, greater dispersal abilities (Davis, 1982; Brown, 1991), and air-breathing habits, should be superior at recolonizing temporary streams or headwater habitats previously denuded by spates or exposed by droughts. Although no one to our knowledge has directly tested the hypothesis of greater rates of passive dispersal in pulmonates, prosobranchs (with the possible exception of smaller species like hydrobiids) would seem to be restricted to adult dispersal along steam channels, perhaps explaining the greater degree of genetic isolation and endemism in prosobranchs (Davis, 1982).

Physiological factors can influence the distributions of species as well. For example, temporary streams or backwaters without much riparian cover often experience extreme temperatures because of their shallow depths and periodic droughts. Potentially lethal temperature stress, hypoxic conditions, or desiccation are likely in stagnant pools or exposed stream beds during drought conditions. As a result, increased resistance and capacity adaptations to temperature and dissolved oxygen variation should be selected for, as they are in other invertebrates or fish species that are characteristically found in temporary streams (Horwitz, 1978; Matthews, 1988; Moyle, 1988; Miller and Golladay, 1996; Jacobi and Cary, 1996; Williams, 1996). Alternately, if streams arise from springs, variation in physicochemical variables may increase away from spring sources, favoring more tolerant species downstream from springs.

In temporary headwaters, crayfish and other invertebrate predators probably achieve their greatest importance, as they do in temporary, fishless ponds (Lodge *et al.*, 1987). In more permanent lentic habitats, fish predators become a more significant factor (Brown and DeVries, 1985; Bronmark *et al.*, 1992), and similar trends occur in higher order lotic habitats as well (Horwitz, 1978). For example, in a Kentucky stream, fish diversity increased from one species in a headwater stream to 15 in a third-order river (Lotrich, 1973), and nearly 150 fish species exist in the Ohio River (Pearson and Krumholz, 1984). The fraction of predatory fish also increases with stream order (Moyle, 1988). Therefore, in headwaters or other temporary lotic habitats we predict that responses to low predation pressure should be only behavioral, for example, crawling out of the stream (Alexander and Covich, 1991a, b; Covich *et al.*, 1994). Morphological defenses (thick and sometimes knobby shells) would be expected to occur in large, unglaciated rivers, both because fish prefer thin-shelled species (Stein *et al.*, 1984) and because of the longer evolutionary time for selection of morphological defenses (Vermeij and Covich, 1978; Vermeij, 1987).

Considerable experimental evidence exists that predators can control the structure of lentic gastropod assemblages (Brown and DeVries, 1985; Sheldon, 1987; Kesler and Munns, 1989; Bronmark *et al.*, 1992; Lodge *et al.*, 1994). Comparatively little information about lotic assemblages is available, although predators clearly affect at least some gastropod populations in streams (Crowl, 1990; Crowl and Covich, 1990; Covich *et al.*, 1994).

In regard to relative competitive ability, some experimental evidence suggests pulmonates could be better competitors, at least in lentic systems, because they possess smaller, more numerous radular teeth and can efficiently remove adnate periphyton, whereas the prosobranch studied only removed overlying filamentous algae (Barnese *et al.,* 1990). The argument that the type and amount of food resources available determine gastropod distributions implicitly assumes that resources are limiting and intra- and interspecific competition are, therefore, more likely. Considerable debate has occurred on the role of competition in structuring communities (Strong *et al.*, 1984), but two literature reviews found substantial empirical evidence for competition (Connell, 1983; Schoener, 1983). Likewise, a recent statistical meta-analysis combining a large number of empirical studies (Gurevitch *et al.*, 1992) supported a role for competition, even in herbivores, contrary to the Hairston-Smith-Slobodkin hypothesis (Hairston *et al.*, 1960).

Several direct or indirect observations suggest that competition could occur among snails: (1) dense gastropod assemblages do limit their food resources (Sheldon, 1987; Osenberg, 1989; Bronmark *et al.*, 1992; Hill *et al.,* 1992; Rosemond *et al.,* 1993); (2) species diversity increases with increasing habitat complexity (Harman, 1972; Brown and Lodge, 1993); and (3) field sampling surveys suggest that fewer congeners coexist than expected by chance (Harman, 1968; Harman and Berg, 1971; Dillon, 1981, 1987). Brown (1982) experimentally demonstrated competition between two vernal pond snails. These populations occurred in ephemeral ponds, but because significant niche overlap occurred in only one of six possible pair-wise combinations of species, interspecific competition was probably not a structuring force in these fluctuating lentic habitats. However, in more permanent habitats like lakes or large rivers that have more diverse assemblages, competition might be more likely. For example, up to 14 gastropods are often found in macrophyte beds in lakes in northern Wisconsin (Brown and Lodge, 1993).

In Table 1, we list four specific hypotheses about important regulating factors, and our prediction in each case of the relative abilities of pulmonates and prosobranchs in coping with the factor, based on the existing knowledge of gastropod ecology outlined above. It is our hope that these hypotheses will form the basis for understanding the distributional ecology of gastropod assemblages, especially those that contain threatened or endangered species.

Table 1. Specific hypotheses and predictions on ecological differences between pulmonates and prosobranchs, and how these differences can affect distribution patterns.

Hypothesis 1: Relative competitive abilities are important in determining lotic gastropod distributions.

Predictions

P_1 - Pulmonates, with finer radular teeth, are better at removing adnate algae, giving them a competitive advantage when food is a limiting factor.

P_2 - *Elimia* and other prosobranchs are poorer competitors if food is a limiting factor.

Hypothesis 2: Disturbance events like spates and the relative dispersal/colonization abilities of gastropods are important factors affecting snail distributions.

Predictions

P_1 - Pulmonates are better dispersers (*e. g.* greater passive dispersal by birds and greater locomotory rates) and are suited to headwaters with high frequencies of spates.

P_2 - Prosobranchs (with the possible exception of *Elimia*) are poorer dispersers and less resilient to spates.

Hypothesis 3: Physiological adaptations are important in determining gastropod distributions.

Predictions

P_1 - Pulmonates are most tolerant to extreme physical and chemical variables (*e. g.* temperature, hypoxia, and dessication) making them better adapted to temporary headwaters and shallow littoral areas.

P_2 - *Elimia* and other prosobranchs are less tolerant to extreme temperature and hypoxia but survive as well in middle reaches where flow is sustained.

Hypothesis 4: Predation pressure increases with river order and is a significant factor determining snail distributions.

Predictions

P_1 - Pulmonates are most susceptible to predation because of their thin shells, and are restricted to headwaters or shallow littoral zones where fewer predators occur.

P_2 - Prosobranchs are less susceptible to predation and can co-occur with abundant and diverse predator assemblages (both fish and crayfish) of larger streams.

We also present data that bear on at least some of these hypotheses. Specifically, we sampled one river system, the Salt River in western Kentucky, to determine if there were any longitudinal differences in the distributions of pulmonates and prosobranchs. To gauge differences in in-stream dispersal among several pulmonate and prosobranch species, we measured locomotory rates and righting responses in laboratory experiments. Crawling rates are obviously related to dispersal within streams; we consider righting responses to be important as well because detached snails would be more susceptible to being washed downstream. An artificial stream was used to conduct a month-long competition experiment between one pulmonate common in headwaters of the Salt River and one prosobranch common in the Ohio River. We evaluated data in the literature on the physiological adaptation of both groups to temperature and dissolved oxygen extremes. Finally, we discuss existing work on differential predation risk of pulmonates and prosobranchs (done mostly with lentic species) and present some data on relative vulnerability to crayfish of a head-water pulmonate found in the Salt River and a prosobranch species common throughout much of the river system.

METHODS

Habitat description and sampling methods

The Salt River is a tributary system of the Ohio River located east and south of Louisville, Kentucky, with an average annual discharge of 51 m^3/sec (based on 6 years of data, United States Geological Survey, Kentucky Water Division). The headwaters of the river originate on the flat, forested lands east of Louisville in small marsh-like springs, and these headwaters often dry late in the hydrological cycle in summer and fall. The higher order creeks in the system are often deeply incised into the karst topography of the area, with very little littoral zone habitat. The Salt River watershed includes large tracts of relatively undisturbed eastern deciduous forest, but some branches of the river drain agricultural and suburban areas near Louisville. The riparian zone is typical of lowland, eastern deciduous forest streams (but also includes some disturbed, open canopy areas), and substrata vary from limestone cobble in headwaters to a clay/silt bottom in downstream reaches.

We collected data on relative gastropod abundance in both fall 1994 and spring 1995 from headwaters to large-order sites of the Salt River. Our approach was to sample vegetation, leaf debris, and erosional limestone habitats using kick-nets and visual searching (*e. g.* turning over rocks, leaves, and snags) for at least 30 min at each site, or until continued sampling suggested no additional changes in the relative abundance of snail species. We explicitly avoided collection sites near urban or agricultural influences, to minimize problems with human alterations of the streams. All snails collected were fixed in 70% ethyl alcohol and were later identified using keys by Burch (1982), Branson (1987), and Brown (1991). We noted the number of sites, in several categories of river orders, in which each species was found, as well as the total number of individuals collected. We used a G-test of independence to determine if the relative abundance of prosobranchs was independent of river order.

Relative dispersal abilities

To be conservative, we considered only differences in in-stream dispersal ability between the two groups. In a series of laboratory experiments (winter to spring 1995), we measured differences in two behaviors that should be related to in-stream dispersal ability: turnover times (*e. g.* righting responses) and rates of movement. To measure the first variable, we recorded the time necessary for individuals to right themselves after being detached from the substratum (N = 17-38 individuals, depending on species). Solitary animals were placed in Syracuse dishes (5 cm high x 10 cm diameter) in 200 ml of standard reconstituted freshwater (hard water type; APHA, 1985) at room temperature (22-26°C). The four species with spiral shells (*Physella, Elimia, Pleurocera*, and *Lithasia*) were turned over onto the dorsal sides of their shells. Because of its sinistral, planospiral shell, *Helisoma* was turned over on its right side. Each snail was lightly prodded (with a blunt probe) to withdraw into its shell, or to release any air in the pulmonate mantle cavities which would cause the snail to float. The time necessary to extend the foot and reattach to the substratum was recorded.

Movement rates were recorded (N = 31-42 individuals, depending on species) by placing solitary animals in clear plastic chambers (26 x 16 x 10 cm high) in 800 ml of standard reconstituted freshwater at room temperature. After allowing 300 s for habituation, the snail's movement rate across the container was recorded on a 2 cm gridded surface for 300 s. In both cases, we contrasted differences among species with a one-way analysis of variance.

Competition experiment

To gather evidence on competitive abilities, we performed a month-long (September 1994) experiment in artificial stream mesocosms at Louisville Water Company's Payne water treatment facility. The artificial streams received filtered (large debris removed) water and sediment from the Ohio River. The stream channels were shaded, rectangular polyvinyl chloride troughs (10 cm depth and width x 3 m length). Water level (1 cm at the inflow, 7 cm at the outflow) was maintained by a computerized pump system attached to a header tank. Outflows were covered with 1 mm mesh weirs to contain the snails. Streams were not stocked with artificial substrata or periphyton, but they did develop a silt substratum ranging from 1 to 3 cm with rich coverings of filamentous green algae.

We selected two species from separate ends of our river gradient, *Helisoma trivolvis* (a pulmonate found in headwater streams, see Results) and *Lithasia obovata* (Say, 1829) (a large river prosobranch) and maintained them alone and in mixed populations in an additive design (Snaydon, 1991). Densities of 30 individuals per species per channel were chosen to be representative of field densities, based on sampling data (K. Greenwood, University of Louisville, unpubl. data), and six replicates were completed in each of the three (two mono-specific and one mixed) treatments. Each mono-specific treatment used 30 snails, and the mixed treatments used 30 of each species. Although total snail biomass does vary among treatments in such an additive design (versus the substitutive design with total numbers held constant in all treatments), additive designs are considered superior because they do not confound the effects of intraspecific and interspecific competition (see detailed arguments by Snaydon, 1991). By comparing the reduction in growth of each species under mixed conditions, relative to each other, one can still judge relative competitive abilities. We also selected these two species because they are of roughly the same individual biomass, with *Lithasia* slightly larger (mean wet mass = 305 mg ± 20 mg SE) than *Helisoma* (217 ± 20 mg). Each snail was weighed (± 1 mg) before and after the experiment to determine the impacts on growth (increase in mean individual biomass per stream channel). To test for differences in growth, we performed a two-way analysis of variance (two species times alone versus mixed) on per capita growth rate, using the stream channel as the experimental unit.

Predation experiment

Little is known about the relative vulnerabilities of gastropods to invertebrate predators. Because of this, we performed laboratory experiments on selectivity of the crayfish *Orconectes virilis* (Hagen, 1817), which is quite abundant in the Salt River drainage. Overnight trapping in early October 1994 suggested high abundance (mean individuals/trap = 26.4 ± 5.7 SE, N = 8) in Guist Creek, a fourth-order tributary of the Salt River located 50 km east of Louisville. These trapping rates were greater than those in Wisconsin lakes where crayfish predation was experimentally shown to control snails (Lodge *et al.*, 1994). Trapping rates could have been high because October is the low point in the river drainage's hydrological cycle. Guist

Creek was essentially a series of disconnected pools, further concentrating the crayfish.

Crayfish were held overnight in 40 l aquaria with brick refuges before being used in experiments. Our experiments used methods developed by Alexander and Covich (1991a, b) and Covich *et al.* (1994). A single, male crayfish (mean carapace length = 40.8 mm ± 0.7 mm SE) was held in a 2 l aquarium overnight with 25 each of adult *Physella heterostropha* (Say, 1817) and *Elimia semicarinata* (Say, 1829). Experiments were completed at room temperature with 1 l of EPA reconstituted water. Numbers of snails escaping vertically above the water line were observed next morning, to determine the fraction of surviving snails actually available to the crayfish. We tested for a difference in crayfish preference for the two species with a paired t-test (Peterson and Renaud, 1989).

RESULTS

Sampling survey

A clear pattern emerged (Table 2) with pulmonates *(Physella heterostropha, Helisoma trivolvis)* found mostly in first- or second-order streams. Physids were found at nine of the 12 sites, and *Helisoma* was found at five of the 12 sites in streams of first or second order, indicating that they were fairly widespread in temporary headwater streams. The prosobranch *Elimia semicarinata*, also common in headwaters, was the numerical dominant in third to fifth order streams: physids were found at three of the 11 sites, *Elimina* at ten of the sites, and *Pleurocera acuta* Rafinesque, 1831, at five of the sites. Along with *Elimia*, pleurocerids *Lithasia obovata* and *P. acuta* were also found in rivers of orders 6 to 9. Finally, at several littoral sites along the Ohio River, *P. canaliculata* (Say, 1821) and *L. obovata* were the dominant gastropods. Thin-shelled species such as *Physella* are extremely rare in littoral zone habitats of the Ohio River, based on our experience (J. Alexander, pers. obs.). A G-test of independence (Sokal and Rohlf, 1981) contrasting pulmonate and prosobranch distributions across the river orders was highly significant (G = 707, P < 0.001), indicating that the two groups were not distributed in the same fashion across river size categories. Based on studies of nearby streams (Branson and Batch, 1983; Johnson *et al.*, 1994), we believe that the trend (*e. g.* replacement of pulmonates by prosobranchs) is common.

Table 2. Numbers of gastropods collected in the Salt River, Kentucky, according to river order of each site. The number of sites (N) where each gastropod was collected are indicated in parentheses.

	River Order			
Species	1 - 2	3 - 5	6 - 9	10 or above
	(12)	(11)	(2)	(3)
Physella heterostropha	219 (9)	13 (3)	0	0
Helisoma trivolvis	42 (5)	0	0	0
Elimia semicarinata	726 (5)	1764 (10)	149 (1)	0
Pleurocera acuta	0	17 (5)	166 (2)	0
Lithasia obovata	0	0	710 (1)	143 (2)
Pleurocera canaliculata	0	0	0	72 (2)

Relative dispersal abilities

An Analysis of Covariance indicated that there were significant differences among species in righting response (species effect $F_{4,139} = 40.9$, $P < 0.0001$), but that size (the covariate) did not have a significant effect (covariate $F_{1,139} = 3.3$, $P = 0.07$). Tukey's *a posteriori* ranges (Fig. 1) indicated that *Physella* had a significantly faster righting response than all other species, and that *Lithasia* was significantly slower than all other species except *Pleurocera*.

Physella heterostropha also crawled at a rate four times faster than the other four species (species $F_{4,139} = 38.8$, $P < 0.0001$), with shell length again having little effect (covariate $F_{1,139} = 0.4$, $P = 0.53$). Tukey's *a posteriori* ranges suggested that the other four gastropods had similar crawling rates (Fig. 1).

Competition experiment

Although *Helisoma* and *Lithasia* grew at different rates in monocultures (species $F_{1,22} = 19.8$, $P < 0.001$; Fig. 2), significant evidence of interspecific effects on growth was present for each species as well (alone versus mixed $F_{1,22} = 40.6$, $P < 0.001$). The competitive effect was quite asymmetrical (*i. e.* interaction $F_{1,22} = 20.6$, $P < 0.001$), with the pulmonate suffering a 92% reduction in growth, versus 55% in the prosobranch.

Predation experiment

The crayfish in the current study strongly preferred *Physella* over *Elimia* (means of 50% consumed versus only 0.7 % of the prosobranchs; N = 11, paired t-test, P < 0.001). Almost two-fifths of the remaining physids had crawled out, and none of the prosobranchs, indicating that predation is still differential, even when vertical escape is possible for the thinner-shelled physid (see also Alexander and Covich,

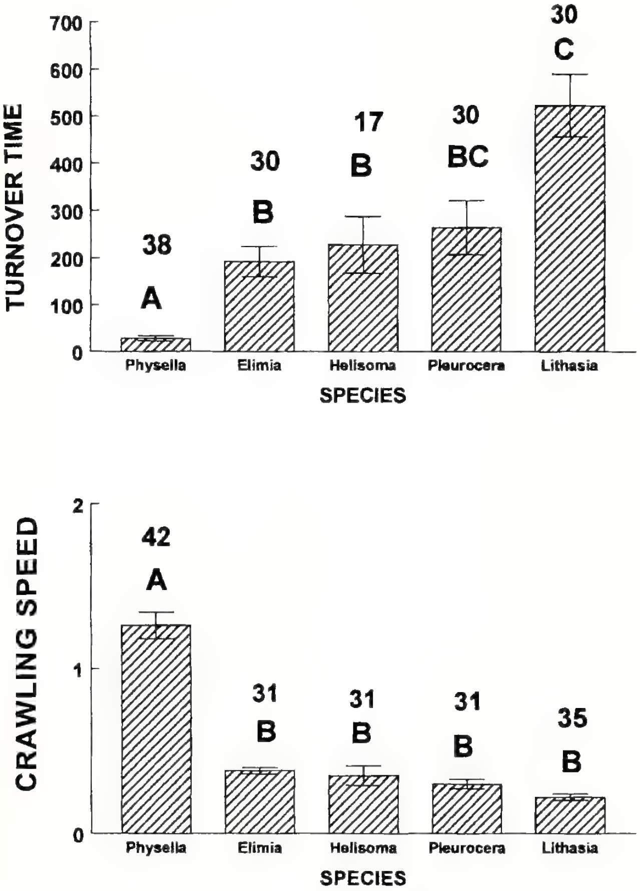

Fig. 1. Comparison of turnover time (righting response time) in seconds (top, mean ± SE, N above histogram) and average crawling speed in millimeters per second (bottom, mean ± SE, N above histogram) for two pulmonates (*Physella* and *Helisoma*) and three prosobranch snails. Means with different letters are significantly different ($P < 0.05$), based on Tukey's *a posteriori* ranges.

1991a, b).

DISCUSSION

Differences in physiological adaptations

A considerable amount of work has been done on the physiological ecology of freshwater snail species, although few comparative studies of lotic species have been undertaken. We therefore summarize the arguments and data presented in reviews of pulmonates (McMahon, 1983) and prosobranchs (Aldridge, 1983). Both authors maintained that pulmonates, which were typically found in shallow, physically and chemically more-variable environments, had broader tolerance adaptations to temperature. However, the ranges reported in field studies were fairly similar (mean range ± SE for pulmonates of 28.2 ± 1.4°C, N = 12 populations, versus a mean range of 26°C for the two prosobranch studies). The mean upper temperature reported for the pulmonates was 30.9°C versus 27°C for the prosobranchs (data presented by McMahon, 1983). However, although the two thermal maxima were similar, pulmonates have been reported in earlier studies to have some of the highest thermal maxima in multicellular animals (Van der Schalie and Berry, 1973; Russell-Hunter, 1978; McMahon, 1983, 1985). For example, physid species are capable of activity under ice cover, and have also been reported in warm springs.

Pulmonates clearly show greater capacity adaptations to changing temperatures. Laboratory studies have suggested that the metabolic rates of pulmonates change less with increasing temperature than those of prosobranchs. The experimental studies, summarized in Table 3, indicate significantly lower Q_{10} values for metabolic rates across the same temperature range ($t = 2.1$, $0.02 < P < 0.05$, $DF = 28$). With Q_{10} values nearer 2.0 (measured in their ambient temperature range), pulmonates can better regulate acute temperature change effects on their metabolic rates (McMahon, 1983). Six of ten pulmonates, versus zero of seven prosobranchs, had Q_{10} values below 2.0. This ability

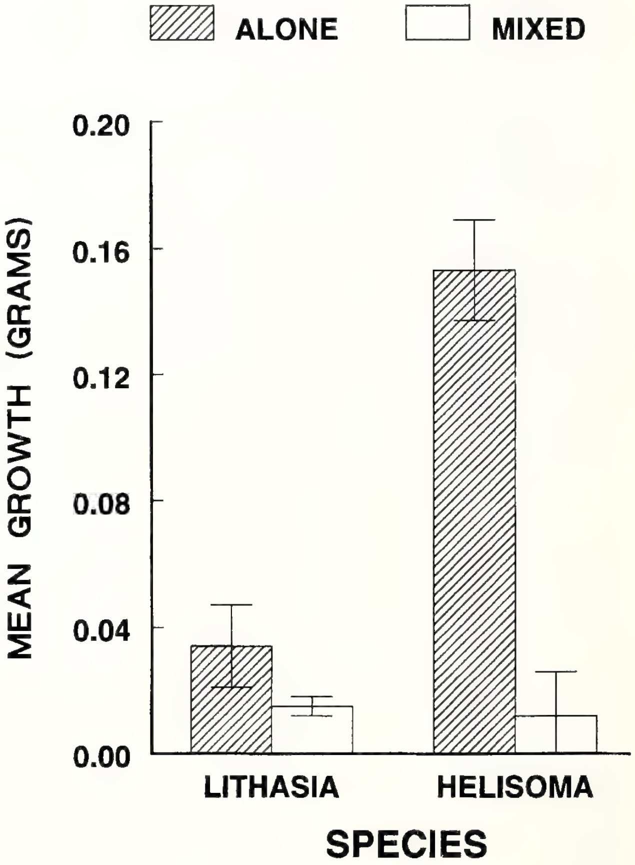

Fig. 2. Results of a month-long competition experiment in artificial streams where each species was reared alone and compared to a mixed population in an additive design. Data are mean increases in individual biomass per stream ± SE, N = 6.

to regulate metabolic rate during acute temperature changes may be an adaptation to life in fluctuating, shallow-water habitats. Prosobranchs may not need metabolic regulation, because they are common in deeper, more permanent, and more thermally stable habitats (McMahon, 1983). McMahon (1983) also pointed out that most pulmonates can reproduce across a broader temperature range than can prosobranchs, again a valuable adaptation in variable aquatic habitats.

Differences between pulmonates and prosobranchs in resistance and capacity adaptations to hypoxia are more complicated. In regard to tolerance, Aldridge (1983) noted that prosobranchs require higher oxygen levels in water than do pulmonates (Boycott, 1936; Palmieri *et al.*, 1980), but this could simply be because pulmonates can use pulmonary (*i. e.* aerial) respiration. Prosobranchs are more resistant to hypoxia in laboratory experiments when aerial respiration is not possible (Von Brand *et al.*, 1950; Hawkins and Ultsch, 1979). There is also variation in resistance to hypoxia within pulmonates. For example, planorbids with their respiratory pigments are more tolerant (and more aquatic) than lymnaeids and physids; the latter two groups rely more on pulmonary respiration and are more amphibious (McMahon, 1983; Alexander and Covich, 1991b; Covich *et al.*, 1994).

Pulmonates clearly have greater capacity adaptations to variation in oxygen concentration, although some background on experimental methods is necessary to illustrate this. In Table 3, the degree of metabolic regulation in response to declining oxygen levels is compared between the two groups by fitting standardized metabolic rate versus PO_2 (partial pressure of oxygen in water) in a quadratic equation (Mangum and van Winkle, 1973), with standardized uptake = $a + b_1(PO_2) + b_2(PO_2)^2$. The quadratic coefficient (b_2, multiplied by 10^3) is the dependent variable, and the degree of metabolic regulation is determined using statistical methods (Mangum and van Winkle, 1973; McMahon, 1985). Values of b_2 (10^3) near -0.1 indicate nearly perfect regulation (the metabolic rate under hypoxia is very near that at air saturation), whereas values near zero indicate conformity, where metabolic rates are a linear function of oxygen concentration (Mangum and van Winkle, 1973). Pulmonates do possess values of b_2 significantly less than those shown by the prosobranchs ($t = -3.15$, $P < 0.01$, $DF = 17$). This result is somewhat surprising (because pulmonates are evidently more sensitive to sustained hypoxia); however this could again reflect their common occurrence in shallow-water environments that are frequently exposed to diurnal cycles of hypoxia.

Table 3. Differences in regulation of metabolic rates as a function of temperature (Q_{10}) and as a function of partial pressure of oxygen (b_2) between pulmonate and prosobranch snails. Data from McMahon (1983). See text for statistical tests of the differences between the two groups.

Group	Mean Q_{10} + SE (N)	b_2 (x 10^3) + SE (N)
Pulmonates	2.12 ± 0.09 (18)	-0.061 ± 0.009 (12)
Prosobranchs	2.76 ± 0.29 (13)	-0.014 ± 0.012 (7)

In summary, pulmonates evidently have smaller tolerance adaptations to long-term hypoxia (they could be able to avoid this problem with aerial respiration) but greater capacity adaptations. Pulmonates are also better adapted to resist desiccation because they can use pulmonary respiration, store their nitrogen excretory products as urea (not ammonia), and can aestivate by forming an epiphragm covering their aperture (McMahon, 1983; Brown, 1991) to minimize water loss.

The fact that pulmonates show smaller changes in metabolic rates to changing temperature or oxygen tension could be critically important in an ecological sense. Gastropods with lower maintenance costs under extreme physical or chemical conditions would have more energy to partition into growth or reproduction, and thus show greater fitness and/or competitive ability. Differences between pulmonates and prosobranchs in shell thickness and reliance on aerial refugia (and aerial respiration) could be contrasting adaptations due at least in part to different life histories and evolutionary pathways in the two groups (Vermeij and Covich, 1978; Alexander and Covich, 1991a, b; Covich *et al.*, 1994).

Literature studies of predation

In Table 4, we have summarized earlier studies of selectivity of fish and crayfish molluscivores in lentic and lotic systems. In the two studies of molluscivorous sunfish (Stein *et al.*, 1984; Klosiewski, 1991), there was a clear preference for thin-shelled pulmonates like *Physella* or *Helisoma* over prosobranchs like *Elimia* or *Campeloma*. Experimental results on selectivity of orconectid crayfish in Wisconsin lakes (Brown, in press) also indicated thin-shelled species like *Physella* and *Gyraulus* are consumed

Table 4. Summary of earlier studies of selectivity among molluscivores.

Predator	Habitat	Preference	Reference
Red-ear sunfish	Ohio rivers	*Physella* > *Helisoma* > *Elimia*	Stein *et al.*, 1984
Pumpkinseed sunfish	Wisconsin lakes	*Lymnaea* > *Amnicola* > *Campeloma*	Klosiewski, 1991
Orconectes spp.	Wisconsin lakes	*Gyraulus* > *Physella* > *Amnicola*	Brown, in press

more frequently than *Amnicola* spp.

Evaluation of hypotheses regarding pulmonates and prosobranchs

The results of sampling the Salt River support our predictions of where pulmonates and prosobranchs should occur. Specifically, the lack of thin-shelled species like *Physella* in large rivers could be due to a greater diversity, abundance, or greater body size of fish predators, whereas thick-shelled species (*e. g. Pleurocera canaliculata*) could be absent from from the more temporary headwater streams due to desiccation or hypoxia resulting from low or intermittent flow, or because of relatively poor dispersal abilities. Pulmonates might also be feeding generalists or have more success at repopulating disturbed sites because of their "r-selected" life histories. Although pulmonates as a group are considered micro-herbivores, *Physella* and *Helisoma* are feeding generalists (Brown, 1982, 1991).

Some aspects of these distribution patterns, however, could be unique to the Salt River system. Little shallow-water macrophyte habitat occurs along the Salt River because of the constrained nature of the littoral zone, a function of the river eroding a steep channel in the limestone substratum. In river systems with extensive marsh-like littoral zones, pulmonates could be more common along the edges of rivers. However, we do not consider this type of horizontal distribution pattern in rivers as repudiating our general hypothesis. These littoral macrophytes might provide shelter from predators, and shallow littoral habitats are again expected to vary more in physicochemical variables than the main river channel. Similarly, many southeastern U. S. rivers originate from springs, or have montane headwater reaches that are quite turbulent. In these systems we might actually expect a preponderance of prosobranchs in headwaters, because turbulence increases dissolved oxygen levels, or because spring sources vary little in physicochemical variables (see data in Johnson *et al.*, 1994). Pulmonate species might become more common downstream, because of the addition of more ephemeral littoral zone habitats, or greater variation in physicochemical variables (P. Johnson, Southeastern Aquatic Research Center, unpubl. data.; Foin and Stiven, 1970; Dillon and Benfield, 1982). Again, such distributional patterns would not contradict our general hypothesis. Finally, the prosobranch *Elimia* was common in both the headwaters and middle reaches of the Salt River, contrary to our prediction that pulmonates should dominate headwaters. Perhaps those particular headwaters are less susceptible to drying.

The experimental results and literature review also suggest support for some of our hypotheses. An exception is that the prosobranch, *Lithasia*, a common denizen of higher-order rivers, appeared to be a better competitor than the headwater pulmonate, *Helisoma*. Growth declined less under interspecific competition in this large-river prosobranch than in the pulmonate. The result is again somewhat surprising in light of earlier research suggesting that pulmonates, as a group, crop periphyton resources more closely to the substratum (Barnese *et al.*, 1990). However, the earlier study was completed in a lentic habitat, and only one species of prosobranch, *Elimia livescens* (Menke, 1830), was compared to several pulmonates. Also, there is considerable variation *within* pulmonates in grazing ability. For example, lymnaeids are, because of differences in radular anatomy, better adapted to removing filamentous algae than are physids, whose complex teeth are better at removing adnate diatoms (see references by Brown, 1991). Thus, differences in grazing ability could depend on the particular pulmonate or prosobranch family in question.

Because *Lithasia* is most common on littoral-zone cobble along the Ohio River, we would not have a *priori* predicted it would do better than the pulmonate, which occurs in soft sediments such as those lining the bottom of the experimental channels. We would have also expected that the relatively short time-span of the experiment would have favored the pulmonate, because of its short life cycle. However, it is also important to note that *Lithasia* and *Helisoma* do not overlap in nature, being at opposite ends of the river continuum, and thus lack an extensive history of competition.

The evidence for dispersal ability as a limiting factor in field distributions is equivocal. *Physella* clearly is the most active species and disperses at a higher rate, as expected because it is most common in headwater environments. However, *Helisoma*, which is also common in the same environments, moves no faster than any of the prosobranch species, including *Lithasia* and *Pleurocera*, which are found only in medium to large rivers. Of course, both pulmonates could have greater passive dispersal abilities, as mentioned earlier, a factor which we did not investigate, and pulmonates can also self-fertilize, a valuable aid in colonizing new habitats.

The available data in the literature do seem as a whole to indicate that pulmonates are better adapted physiologically to the large range of physical and chemical variation occurring in ephemeral habitats like shallow littoral zones, or the seasonal headwater habitats in the Salt River. Conventional wisdom is that pulmonates show broader resistance and capacity adaptations to temperature. The capacity adaptations of pulmonates to variation in dissolved oxygen are at least as great as in prosobranchs, and pulmonates can respire aerial oxygen. Pulmonates are also better at resisting desiccation. In addition, prosobranchs do not have the ability to aestivate and survive over periods when habitats dry.

Finally, both our experimental results and the literature clearly suggest the importance of predation as an ecological factor determining lotic gastropod distributions. As stream order increases, it is likely that the abundance of crayfish (and probably other invertebrate predators) at first increases, and then decreases as fish become more abundant and diverse (Crowl, 1990). The thick shells of prosobranchs afford them better protection from fish (Stein *et al.,* 1984; Klosiewski, 1991) and crayfish predation (this study). They also have an advantage against other shell-invading invertebrate predators like leeches because they possess an operculum (Bronmark and Malmqvist, 1986; Brown and Strouse, 1988).

Suggestions for future research

This paper clearly demonstrates the need for further work on the community ecology of lotic gastropods. It has been our purpose to establish several hypotheses (that are not necessarily mutually exclusive) explaining gastropod distributions along river continua, rather than to definitively identify which hypotheses are most important. For example, future studies could examine the competitive ability of all species in the replacement series in a particular river system to determine, for example, if the competitive hierarchy is transitive. These studies should also gauge the relative importance of intraspecific competition (*e. g.* density dependence) and interspecific competition for both snail groups. Although such a comparative study might prove difficult, future researchers could also test the differential passive dispersal hypothesis by sampling birds and other vertebrates arriving at lotic sites to determine the relative abundance of phoretic juvenile gastropods. Future physiological research should test for differences in capacity and tolerance adaptations among co-occurring sets of gastropods found along river continua to rigorously test the conventional wisdom that pulmonates are better adapted to physically more extreme habitats. Also, although comparative work has been done on pulmonates, very little is known of differences in physiological tolerances among prosobranchs. In addition, little is known of the comparative tolerances of pulmonates and prosobranchs to other potentially important physiological stresses occurring at different points along the river continuum, such as spates, turbidity, and wave action.

We hope that this paper will stimulate future research in understanding the community ecology of riverine snail assemblages, and thus help preserve the rich gastropod assemblages found in many southeastern U. S. river systems. This is especially important because as many as one-third of the species face threats of extinction (Neves *et al.*, 1998). Although they are relatively unstudied, prosobranch gastropods like *Elimia* are important as grazers in southeastern U. S. lotic systems (Richardson *et al.*, 1988; Hill *et al.*, 1992; Rosemond *et al.,* 1993). A vital first goal for conservation efforts is to more fully understand what ecological and evolutionary factors constrain the ranges and population biology of these lotic gastropods.

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An analysis of the community structure of subtidal and intertidal benthic mollusks of the Inlet of Baño (Ría de Ferrol) (northwestern Spain)

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Abstract: A study of the benthic communities of the Inlet of Baño (Ría de Ferrol) (northwestern Spain) was carried out, encompassing the examination of 75 sampling stations (35 subtidal and 40 intertidal). Data were subjected to classification and ordination techniques. Two major assemblages were identified, and divided into five subgroups in terms of dominance of the species, constancy, and fidelity. The subtidal zone was characterized by the *Abra alba* (Wood, 1802) community. It was structured in facies as follows: The *Nucula nitida* Sowerby, 1833-*Thyasira flexuosa* (Montagu, 1803) facies, was the innermost, in the eastern area of the inlet; another facies existed in the transition toward the *Clausinella fasciata* (da Costa, 1778) community in the outer inlet area. In the intertidal zone the *Cerastoderma edule* (Linné, 1758)-*Scrobicularia plana* (da Costa, 1778) community was defined, which was also structured in facies: one facies, located at the mouth of the river, was dominated by *Hydrobia ulvae* (Pennant, 1777); another facies, found in the inner inlet area associated with a meadow of the seagrass *Zostera noltii* Hornem., 1832, characterized by *Bittium reticulatum* (da Costa, 1778), *Loripes lacteus* (Linné, 1758), *C. edule*, *Venerupis senegalensis* (Gmelin, 1791), and *Rissostomia membranacea* (Adams, 1800), and a third facies, situated at the border of the inlet, included *Gibbula umbilicalis* (da Costa, 1778), *Littorina littorea* (Linné, 1758), *L. obtusata* (Linné, 1758), *V. senegalensis*, etc., as dominant species. The analyses showed that sediment parameters (mainly grain size), organic matter in the subtidal stations, and grain size and depth are the most important factors governing the distribution and abundance of the communities.

Key words: community structure, intertidal, subtidal, mollusks, Ría de Ferrol, Spain

Research on the macrobenthic communities of the northern coast of the Iberian Peninsula has been carried out by several authors, who have taken both a faunistic and an ecological-descriptive approach. Their sources of information have been primarily articles on the ecology of the macrobenthos, and their ultimate goals have been to carry out analyses of the faunistic community as a whole. This is reflected by the great number of biocenotic studies on the benthos living on soft substrata in recent years on the Galician coasts (Viéitez, 1976, 1981; Anadón, 1980; Mora, 1980; Penas and González, 1983; Rodríguez Castelo and Mora, 1984; Planas and Mora, 1984 a, b; Laborda, 1986; Planas, 1986; López Serrano and Viéitez, 1987; Viéitez and Baz, 1988; Junoy and Viéitez, 1989, 1990; Mazé *et al.*, 1990; Palacio *et al.*, 1991, 1993; Currás and Mora, 1991, 1992; Pérez Edrosa and Junoy, 1993).

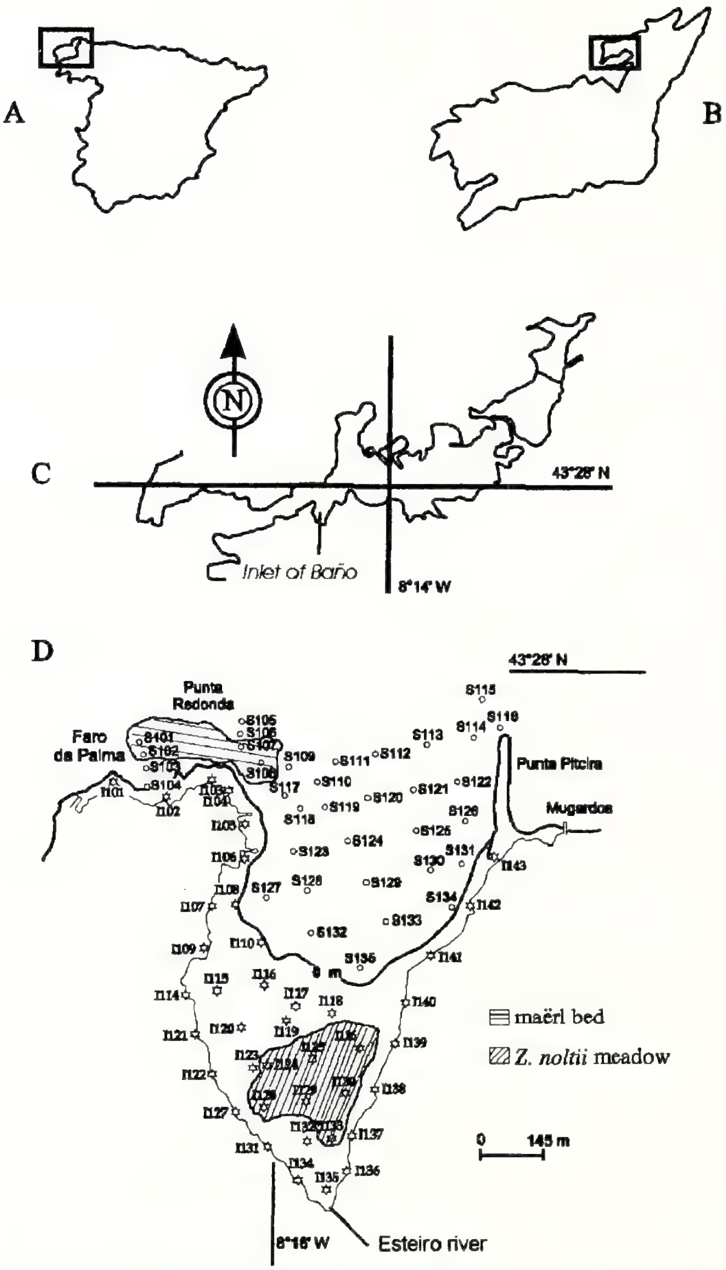
Frequent studies have also been made on the malacological communities of different geographical areas of the European Atlantic coasts (Lande, 1975; Evans and Tallmark, 1976; Petersen, 1977; Glémarec, 1978; Tunberg, 1981; Dewarumez, 1983; Gentil *et al.*, 1986; Cornet, 1985; Quintino *et al.*, 1986; Sauriau *et al.*, 1989).

However, despite the plethora of faunistic studies involving Galician mollusks, there is a need for research on their sinecology. An exception to the lack of sinecological studies is the work by Cadée (1968) in the Ría de Arousa. Therefore the purpose of this work was to study the ecology of the benthic populations of mollusks living on soft substrata, both subtidal and intertidal, of the Inlet of Baño (Ría de Ferrol, northwestern Spain). The aim was to define the different communities living in the inlet and how they relate to physicochemical factors (granulometry, sorting coefficient, organic matter, carbonates, and nitrogen).

MATERIAL AND METHODS

STUDY AREA

The Inlet of Baño (Fig. 1) is located on the southern border of the central channel in the Ría de Ferrol between the Punta do Faro da Palma (43°27'52"N; 08°16'49"W) and Punta Piteira (43°27'57"N;


A
B
C
N
43°28' N
Inlet of Baño
8°14' W
D
43°28' N
Punta Redonda
Faro da Palma
Punta Pitcira
Mugardos
S101
S102
S103
S104
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S106
S107
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I131
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I134
I135
I136
I137
I138
I139
I140
I141
I142
I143
9 m
maërl bed
Z. noltii meadow
0 145 m
8°16' W
Esteiro river

Fig. 1. Location of the 75 sampling stations in the Inlet of Baño (Ría de Ferrol), northwestern Spain. A. Spain. B. Galicia. C. Ría de Ferrol. D. Inlet of Baño. (I, intertidal station; S, subtidal station).

08°15'37"W), with an area of 0.5 km2 and a maximum depth of 18 m (Fig. 2). The inlet is oriented in a NNE-SSW direction; the prevailing winds are southwesterly for most of the year, except in summer when northeasterlies become dominant. The mean tidal range in the ria is 2.7 m, and tidal effects give rise to strong currents (up to 1.5 m/s in the ria's central channel). Outward movement of water from the ria provokes flow to the southeast within the inlet, while movement into the ria provokes flow to the SSW within the inlet. These currents, which are stronger at the mouth of the inlet than at more distal points, are the dominant factor affecting sediment distribution within the inlet (Fig. 3).

The inlet is characterized by soft bottom bordered by a rocky strip. A maërl bed is present in four of the western stations, between 4.5 and 10.5 m depth. A seagrass meadow of *Zostera noltii* Hornem., 1832, is present in the central intertidal zone.

The distribution of different grain sizes suggests an

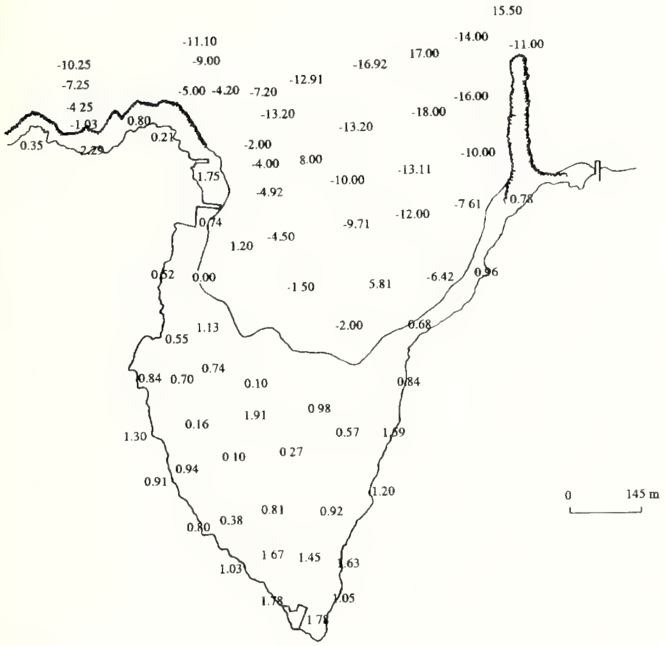
15.50
-11.10
-9.00
-14.00
-11.00
17.00
-16.92
-10.25
-7.25
-12.91
-5.00 -4.20 -7.20
-16.00
4.25
-13.20
-18.00
-1.03
0.80
-13.20
0.35
2.29
0.21
-2.00
-10.00
-4.00
8.00
-13.11
1.75
-10.00
-4.92
-7.61
0.78
-12.00
0.74
-9.71
-4.50
1.20
0.62
0.00
5.81
-6.42
0.96
-1.50
-2.00
0.68
1.13
0.55
0.74
0.84 0.70
0.10
0.84
0.98
1.91
0.16
0.57
1.69
1.30
0.10
0.27
0.94
0.91
1.20
0
145 m
0.81
0.92
0.80
0.38
1.67
1.45
1.63
1.03
1.05
1.78
1.78

Fig. 2. Depth (m) of the subtidal (-) and intertidal (+) stations.

enrichment gradient for fine fractions on the eastern part of the Inlet of Baño. Sandy muds prevail in this area, while the coarsest sediment fractions are prevalent in the outer part. On the other hand, the percentages of organic matter are low, with enrichment toward the eastern part (Table 1). Nitrogen follows a variation pattern similar to that of organic matter. Calcium carbonate does not present a high percentage, with highest values found in the subtidal zone (Olabarria *et al.*, 1996).

The area has a temperate humid Atlantic climate and the hydrographic conditions show thermal variations of more than 5°C in the first 10 m. This variation decreases with depth. Salinity fluctuates with the tides and seasons, due to the fact that the ria has difficulty in draining because of the narrow central channel, which creates water masses having different salinities. The pH values are consistently between 7 and 9, and there are no anomalous data that would indicate a source of excessive acidity or alkalinity (COTOP, 1987).

SAMPLE COLLECTION

The sampling program, which was designed to provide sufficient information on the distribution of the different species of mollusks, consisted of 35 subtidal and 40 intertidal stations sampled from July 1991 to June 1992. Sampling points were selected along 12 parallel transects across the inlet at 100 m intervals, taking samples at the points that were judged by visual examination to show a change in nature, texture, or substrate covering. Intertidal samples were additionally collected at the ends of each transect and, in the inner intertidal zone, samples were also taken every 100 m along each transect (Olabarria *et al.*, 1996). In the subtidal zone, samples were collected by scuba diving. At each point, a 0.5 m^2 square sample was taken, to a depth of approximately 20 cm, using a rectangular shovel. All samples were subsequently wet-sieved through a series of sieves with 10, 2, and 0.5 mm mesh. Finally the sieved samples were transported to the laboratory, and the living specimens were sorted by the remounting technique (Ros, 1975). Sediment samples were obtained for granulometric study. This consisted of an analysis of grain size, organic matter, nitrogen, and carbonates (Guitian and Carballas, 1976).

DATA ANALYSES

Data were organized into station by species matrices. The Shannon-Wiener and Pielou's evenness indices (Washington, 1984) were used to assess species diversity and evenness.

The data for the population studies were processed in two ways: (1) Analyses of qualitative data (presence-absence) of the species in the sampling stations were performed by applying the point correlation coefficient (Φ; Daget, 1976), whereby a correlation matrix is created, followed by classification into clusters through the clustering algorithm UPGMA, by means of the NTSYS-pc program

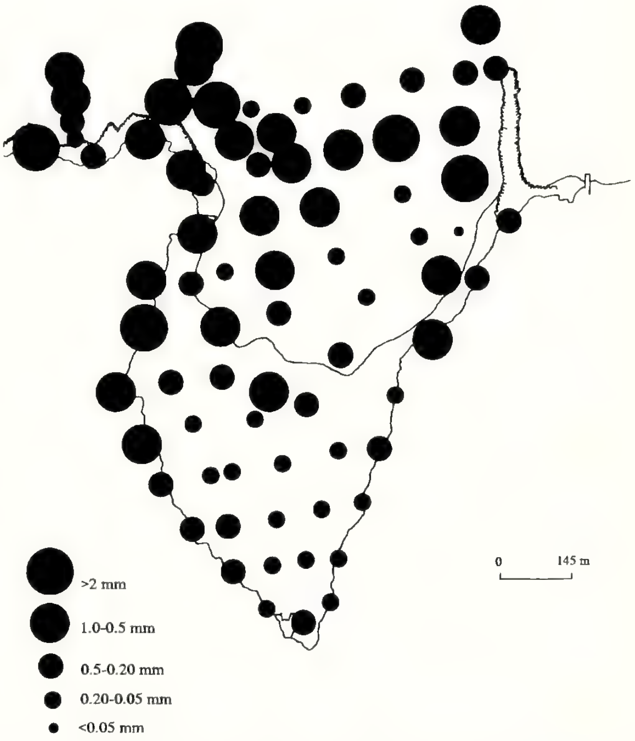
0
145 m
>2 mm
1.0-0.5 mm
0.5-0.20 mm
0.20-0.05 mm
<0.05 mm

Fig. 3. Sediment characteristics of the Inlet of Baño (Ría de Ferrol). Mean grain size in mm.

Table 1. Organic matter (MO), carbonates (CA), nitrogen (N) and sort coefficient (So; Trask, 1932) in subtidal and intertidal stations.

Station	MO	CA	N	So	Station	MO	CA	N	So
S101	0.42	30.22	0.05	2.42	**I103**	0.59	19.64	0.08	> 2.50
S102	0.51	31.11	0.04	2.69	**I104**	0.43	20.57	0.04	1.80
S103	0.42	33.33	0.03	1.76	**I105**	0.67	9.43	0.06	1.58
S104	1.03	18.22	0.06	6.06	**I106**	0.39	12.86	0.03	2.58
S105	0.70	34.67	0.08	4.80	**I107**	0.55	2.57	0.04	2.55
S106	1.04	8.89	0.01	8.36	**I108**	0.54	2.10	0.06	1.95
S107	1.99	32.44	0.13	6.44	**I109**	0.66	1.71	0.06	> 1.65
S108	1.16	34.67	0.10	2.18	**I110**	2.00	8.57	0.06	3.71
S109	1.53	29.33	0.10	9.60	**I114**	0.23	1.71	0.02	> 1.29
S110	0.69	32.89	0.05	2.75	**I115**	0.47	1.03	0.03	1.79
S111	1.69	32.00	0.13	0.18	**I116**	1.41	0.34	0.10	5.07
S112	1.13	13.78	0.09	21.70	**I117**	0.63	26.67	0.08	3.77
S113	0.97	13.78	0.08	2.54	**I118**	0.34	16.29	0.05	2.92
S114	1.37	21.33	0.07	20.99	**I119**	0.59	0.86	0.01	1.63
S115	0.44	34.67	0.04	2.00	**I120**	0.94	0.34	0.02	1.49
S116	0.58	34.67	0.04	1.78	**I121**	0.91	0.86	0.07	> 2.50
S117	0.32	4.89	0.03	2.08	**I122**	1.37	1.71	0.09	1.54
S118	1.55	24.89	0.09	9.57	**I123**	0.64	0.26	0.03	1.89
S119	0.44	17.33	0.03	2.63	**I124**	0.83	0.17	0.04	1.45
S120	0.59	18.40	0.04	3.39	**I125**	0.83	0.26	0.04	1.37
S121	0.87	27.56	0.08	2.54	**I126**	0.43	1.03	0.02	1.27
S122	0.60	30.22	0.07	7.50	**I127**	0.51	0.86	0.04	2.29
S123	0.45	16.44	0.04	2.35	**I128**	0.82	0.34	0.02	2.23
S124	0.69	30.22	0.06	>3.30	**I129**	1.04	0.86	0.09	1.38
S125	0.97	35.56	0.14	10.04	**I130**	1.23	0.26	0.05	0.32
S126	1.31	22.58	0.05	>3.90	**I131**	0.48	0.86	0.04	4.31
S127	1.83	2.22	0.12	>1.84	**I132**	0.72	0.17	0.03	1.41
S128	0.26	6.29	0.04	>3.50	**I133**	0.91	0.34	0.02	1.43
S129	1.80	17.33	0.12	8.48	**I134**	0.91	0.26	0.03	1.32
S130	1.51	25.78	0.11	9.00	**I135**	0.49	1.03	0.02	1.50
S131	2	16.27	0.10	8.50	**I136**	0.44	0.17	0.35	1.77
S132	0.61	1.33	0.04	1.61	**I137**	0.95	0.17	0.04	1.46
S133	2.04	6.22	0.11	5.21	**I138**	0.80	0.17	0.05	3.77
S134	0.93	19.02	0.03	>3.53	**I139**	0.76	0.86	0.07	3.92
S135	0.42	0.89	0.03	1.70	**I140**	0.63	0.86	0.05	7.17
I101	0.17	12.00	0.02	1.13	**I141**	0.69	4.29	0.06	3.08
I102	0.38	1.42	0.01	1.78	**I142**	0.38	24.89	0.03	1.58
					I143	0.78	14.23	0.06	3.65

(ver. 1.60; Rohlf, 1990).

$$\Phi = \frac{ad - bc}{\sqrt{(a + b)(a + c)(b + d)(c + d)}}$$

where a = number of species in both stations; b = number of species in the first station; c = number of species in the second station; and d = species absent in both stations. Φ values ranged from -1 to +1; $p = a + b + c + d$, $\chi^2 = p\,\Phi^2$.

(2) Analyses of quantitative data of the species present in at least 10% of the stations were performed, omitting those which could create "noise" because of their narrow distribution and scarcity. The Detrending Correspondence Analysis (DCA) was applied according to the polynomial method, by means of the CANOCO-pc program (ver. 3.10; Ter Braak, 1988). The data were transformed according to the formula, $x = \log_{10}(x + 1)$. This technique plots the stations along the axes according to similarity in species composition. The axes are often interpreted as environmental gradients whose identity can be analyzed by means of statistical correlations between the location of the stations on the axes and their environmental characteristics (Eleftheriou and Basford, 1989; Junoy and Viéitez, 1990). The environmental variables measured were: depth (positive values for intertidal stations; negative values for subtidal stations), percentages of the different granulometric fractions, organic matter, silt-clay ratio, carbonates, nitrogen, and sort coefficient. These environmental

Table 2. Species classifications according to constancy and fidelity indices. (C_{Aii}, addition of constancies of species A within each population; N_{A1}, number of stations within population where species A exists; N_1, total number of stations within population).

Constancy index		**Fidelity index**	
$C_{A1} = (N_{A1} / N_1) \times 100$		$(C_{A1} / C_{Aii}) \times 100$	
		accidental	< 10%
rare	< 12%	occasional	11-33%
not very common	13-25%	accessory	34-50%
common	26-50%	preferential	51-66%
very common	51-75%	elective	67-90%
constant	76-100%	exclusive	91-100%

factors were correlated with the axes through Spearman's correlation analysis.

Based on the group of stations defined using the point correlation coefficient, the species were classified according to the criteria of constancy and fidelity, based on number of species (Dajoz, 1971; Table 2), and fidelity-dominance product (FxD; Glémarec, 1964), including only species that appeared in at least two stations (Glémarec, 1964; Cabioch, 1968; Lastra *et al.*, 1990; Junoy and Viéitez, 1990; Currás and Mora, 1991).

RESULTS

FAUNISTIC ANALYSIS, SPECIES DIVERSITY, AND ABUNDANCE

The 75 samples analyzed yielded a total of 20,647 individuals belonging to 148 species, in which gastropods were the most abundant (62.4%; Table 3), followed by bivalves (36.7%); chitons and scaphopods were present in small numbers. The gastropods numerically dominated the intertidal zone, outnumbering the bivalves by more than twofold. This is probably caused by the large number of individuals belonging to abundant species such as *Hydrobia ulvae* and *Rissoa parva*. The latter was also present in the subtidal zone in a high number of samples along with *Hinia reticulata, H. incrassata*, and *Onoba semicostata*. There, bivalves were slightly more dominant than gastropods, due to the high number of individuals of *Mysella bidentata*, in addition to *Papillicardium papillosum*, and several species of Anomiidae. The bivalves *Mytilus edulis* and *Venerupis senegalensis* were the most abundant species in the intertidal zone. [Note: The species *Littorina littorea, L. obtusata, L. mariae, Lepidochitona cinerea*, and *M. edulis* are normally associated with hard bottoms. In some cases during this study, small stones were included in the soft sediment samples collected by shovel from the Inlet of Baño. These small stones provided hard substratum for the chitons, and also for attached algae, thus providing food for the periwinkle species.]

The values of the Shannon-Wiener diversity index (Table 4) fluctuated between 0.00 (station I134) and 4.35 (station S112), and evenness ranged from 0.06 (station I135) to 0.94 (station S124). These two parameters were positively correlated ($r = 0.57$; $p < 0.001$).

Diversity correlated positively with carbonate content, percentage of coarse sand, and sorting coefficient, and negatively with depth and percentage of fine sand. Both relative diversity and evenness showed positive correlations with the percentage of coarse sand, percentage of gravel, and the sort coefficient, and a slightly negative correlation with the percentage of fine sand (Table 5).

The lowest population density was recorded at station I135 with 32 ind/m^2, and the highest density at station S111 (6,040 ind/m^2). The mean density in the subtidal zone (1,294.6 ind/m^2) was higher than that in the intertidal zone (931.9 ind/m^2).

Species richness varied widely, with a greater number of species in the subtidal than in the intertidal zone. The lowest values for species richness were found at the intertidal stations near the mouth of the river, due to high abundance of the dominant species *Hydrobia ulvae*. Species richness correlated negatively with the percentage of fine sand and positively with carbonate content, and percentages of coarse sand, gravel, and silt-clay, which explains the increase in species richness at the outer subtidal stations with coarser grain size and higher silt-clay percentages.

Table 3. Number of individuals (ind) and species (sp), and total percentage (%) of individuals in the subtidal and intertidal zones and in both.

	TOTAL			SUBTIDAL			INTERTIDAL		
	ind	sp	%	ind	sp	%	ind	sp	%
Polyplacophora	177	6	0.85	110	6	0.99	67	4	0.72
Gastropoda	1,288	89	62.39	5,206	82	45.95	7,676	41	82.37
Scaphopoda	9	1	0.04	9	1	0.07	0	0	0.00
Bivalvia	7,579	52	36.70	6,003	52	52.99	1,576	32	16.91
TOTAL	9,053	148	100.00	11,328	141	100.00	9,319	77	100.00

Table 4. Faunistic parameters for each subtidal and intertidal station: abundance, species richness (R), Shannon-Wiener diversity index (H'), Pielou's evenness index (J'), and density (ind/m^2).

Station	Abundance	R	H'	J'	ind/m2	Station	Abundance	R	H'	J'	ind/m2
S101	273	31	4.25	0.86	1,092	**I103**	530	29	3.17	0.62	2,120
S102	194	29	3.61	0.74	776	**I104**	249	24	3.46	0.75	996
S103	158	20	3.09	0.72	632	**I105**	162	12	2.28	0.63	648
S104	112	19	3.43	0.81	448	**I106**	165	13	2.60	0.70	660
S105	204	27	3.85	0.81	816	**I107**	481	23	3.11	0.69	1924
S106	924	37	3.74	0.72	3,696	**I108**	154	11	2.37	0.68	616
S107	461	36	3.82	0.74	1,844	**I109**	387	17	3.48	0.85	1,548
S108	1,188	39	3.26	0.62	4,752	**I110**	56	8	2.11	0.70	224
S109	658	38	3.15	0.60	2,632	**I114**	152	17	2.68	0.66	608
S110	260	32	4.07	0.81	1,040	**I115**	12	5	2.12	0.91	48
S111	1,510	45	2.81	0.51	6,040	**I116**	56	16	3.08	0.77	224
S112	365	42	4.35	0.80	1,460	**I117**	274	25	3.21	0.69	1,096
S113	802	49	4.19	0.75	3,208	**I118**	79	18	3.24	0.77	316
S114	553	43	4.24	0.78	2,212	**I119**	10	4	1.85	0.92	40
S115	218	33	4.11	0.81	872	**I120**	24	9	2.43	0.76	96
S116	523	29	2.87	0.59	2,092	**I121**	140	8	2.14	0.71	560
S117	264	19	2.95	0.69	1,056	**I122**	271	17	2.43	0.59	1,084
S118	231	21	2.24	0.51	924	**I123**	143	11	3.06	0.88	572
S119	127	24	3.39	0.74	508	**I124**	343	14	2.11	0.55	1,372
S120	48	15	3.38	0.86	192	**I125**	363	13	1.78	0.48	1,452
S121	85	24	3.00	0.65	340	**I126**	442	13	1.61	0.43	1,768
S122	369	33	2.87	0.57	1,476	**I127**	62	12	2.92	0.81	248
S123	112	22	3.51	0.78	448	**I128**	53	9	2.59	0.82	212
S124	29	15	3.69	0.94	116	**I129**	734	9	0.44	0.14	2,936
S125	96	21	2.46	0.56	384	**I130**	204	13	1.72	0.46	816
S126	337	29	3.58	0.37	1,348	**I131**	199	9	1.38	0.44	796
S127	40	8	2.39	0.79	160	**I132**	388	4	0.20	0.10	1,552
S128	70	16	3.25	0.81	280	**I133**	802	8	0.30	0.09	3,208
S129	82	21	3.84	0.87	328	**I134**	45	1	0.00	—	180
S130	379	33	2.82	0.56	1,516	**I135**	8	3	1.06	0.07	32
S131	126	21	3.57	0.81	504	**I136**	171	1	0.00	—	684
S132	110	10	1.58	0.47	440	**I137**	746	2	0.06	0.06	2,984
S133	205	26	3.28	0.69	820	**I138**	400	11	2.49	0.72	1,600
S134	131	18	2.50	0.59	524	**I139**	56	9	2.83	0.89	224
S135	84	8	1.94	0.65	336	**I140**	20	6	1.94	0.75	80
I101	142	24	3.99	0.87	568	**I141**	146	13	2.21	0.59	584
I102	333	24	3.41	0.74	1,332	**I142**	131	20	3.41	0.79	524
						I143	186	21	3.48	0.79	744

COMMUNITY STRUCTURE

The classification (Fig. 4) that was obtained after applying the point correlation coefficient for 99% significance ($\Phi = 0.29$) showed two large assemblages (A and B) which belong to the intertidal and subtidal stations, respectively. These assemblages were divided into five subgroups: A1, A2, A3, B1, and B2. Subgroup A1 (five stations) located in the inner part of the inlet near the mouth of the river; subgroup A2 (18 stations) included a group of intertidal stations located along the border of the inlet; subgroup A3 (17 stations) included the intertidal stations of the central zone, mostly covered by *Zostera noltii*, which also comprised two subtidal stations; subgroup B1 (ten stations) located in the middle-eastern subtidal zone; subgroup B2 (23 stations) consisted of the outermost subtidal stations which are subjected to stronger hydrodynamism.

A non-parametric Kruskal-Wallis test proved that there were significant differences between assemblages A and B in terms of the percentages of fine sand, carbonates, and silt-clay, the sort coefficient, and diversity ($p < 0.001$), as well as the percentages of coarse sand, gravel, and organic matter ($p < 0.05$). Assemblage B stations had higher mean percentages of gravel, coarse sand, silt-clay, and carbonates than assemblage A. It also surpassed assemblage A in the mean percentage of organic matter, mean diversity value, and sort coefficient. However, assemblage A stations exhibited higher percentages of fine sand than those of assemblage B. The same analysis was later applied to the different subgroups and a multiple comparison test (Conover, 1971) was carried out *a posteriori*, to reveal the

Table 5. Spearman's rank correlations between ordination axes and community index, and environmental variables in Inlet of Baño. Depth (measured in relation to zero tidal level): subtidal stations (-m) and intertidal stations (+m). (H', Shannon-Wiener diversity index; J', Pielou's evenness index; R, species richness; *, $p < 0.05$; **, $p < 0.01$; NS, not significant.).

				Subtidal		Intertidal	
Environmental variables	R	J'	H'	Axis I	Axis II	Axis I	Axis II
Gravel	0.44**	0.33**	0.51**	-0.29^{NS}	-0.14^{NS}	-0.20^{NS}	-0.52**
Coarse sand	0.43**	0.35*	0.52**	-0.39*	0.62**	-0.13^{NS}	-0.64**
Medium sand	-0.15^{NS}	0.04^{NS}	-0.09^{NS}	-0.05^{NS}	0.10^{NS}	-0.08^{NS}	-0.04^{NS}
Fine sand	-0.69**	-0.24*	-0.69**	0.66**	-0.25^{NS}	0.27^{NS}	0.67**
Carbonates	0.78**	0.13^{NS}	0.66**	-0.59**	-0.40**	-0.05^{NS}	-0.67**
Organic matter	0.14^{NS}	-0.19^{NS}	-0.01^{NS}	0.32^{NS}	-0.52**	-0.21^{NS}	0.12^{NS}
Nitrogen	0.41**	-0.03^{NS}	0.25*	0.22^{NS}	-0.47**	-0.04^{NS}	-0.22^{NS}
Silt-clay	0.45**	-0.04^{NS}	0.27*	0.18^{NS}	-0.17^{NS}	0.29^{NS}	0.05^{NS}
Depth	-0.68**	-0.13^{NS}	-0.56**	0.05^{NS}	-0.06^{NS}	-0.39*	0.32*
Sort coefficient	0.44**	0.30*	0.50**	-0.41*	-0.16^{NS}	-0.09^{NS}	-0.41**

groups that significantly differed from one another in the different parameters (Table 6).

The ordination analysis (DCA) was carried out on the subtidal and intertidal stations as a whole. However, because of the excessive concentration of points in the upper and lower quadrant of the negative part of axis I, a separate analysis for the subtidal and intertidal samples was performed. These analyses were based on the groups derived from the application of the point correlation coefficient for a 99% level of significance.

In the analysis of the intertidal stations, the first two axes accounted for 36.4% of the total variance (Fig. 5). Axis I had a slightly negative correlation with the depth parameter, and axis II correlated positively with the percentage of fine sand and negatively with the percentage of carbonates and coarse sand (Table 5). In this analysis there are three groups of stations; on the positive side of axis I, both in the upper and lower quadrants, there is a group of stations that in the similarity (Φ) and constancy-fidelity analyses distinguished subgroup A3. In the upper left-hand quadrant, there was a group of closely-linked stations for which the most characteristic species was *Hydrobia ulvae*. This species reached densities of 2,960 ind/m^2 (station I137). This group defined subgroup A in the similarity analysis. Below, in the upper left-hand quadrant which continued onto the lower quadrant, there was a group of stations and species that characterized what has been defined as subgroup A2 using the point correlation and the constancy-fidelity indices.

In the analysis of the subtidal stations, the coordinates of the first two axes accounted for 33.4% of the total variance (Fig. 6). Axis I had a high positive correlation with the percentage of fine sand and carbonates, and axis II displayed a correlation with the percentage of coarse sand and organic matter, and to a lesser extent with the percentage of carbonates and nitrogen (Table 5). In this analysis, a group of stations and species defining subgroup B1 can be seen in the upper and lower right-hand quadrants, located toward the positive end of axis I. On the negative side of axis I, in both the upper and lower quadrants, there is a group of stations and species that has been defined by the similarity index (Φ) and the constancy and fidelity indices as subgroup B2. In the vicinity of this group there is a group of stations (S126, S122, S112, S118, S130, S134) which are transition stations, grouped in the same cluster in the point correlation analysis (subgroup B2). These are stations with modified bottoms, although originally they most likely had bottoms similar to the stations in subgroup B1. However, due to dredging operations, sediments from other

Table 6. Multiple comparison test (Conover, 1971) between the subgroups derived from the point correlation analysis (A1, A2, A3, B1, B2) indicating groups that showed no significant differences ($p < 0.01$).

Gravel percentage	Coarse sand percentage
A1 < A3 < B1 < B2 < A2	A1 < A3 < B1< A2< B2
Fine sand percentage	**Silt-clay percentage**
B2 < A2 < B1 < A3 < A1	A1 < A2 < A3 < B1 < B2
Carbonates percentage	**Diversity index**
A1 < A3 < A2 < B1 < B2	A1 < A3 < A2 < B1 < B2

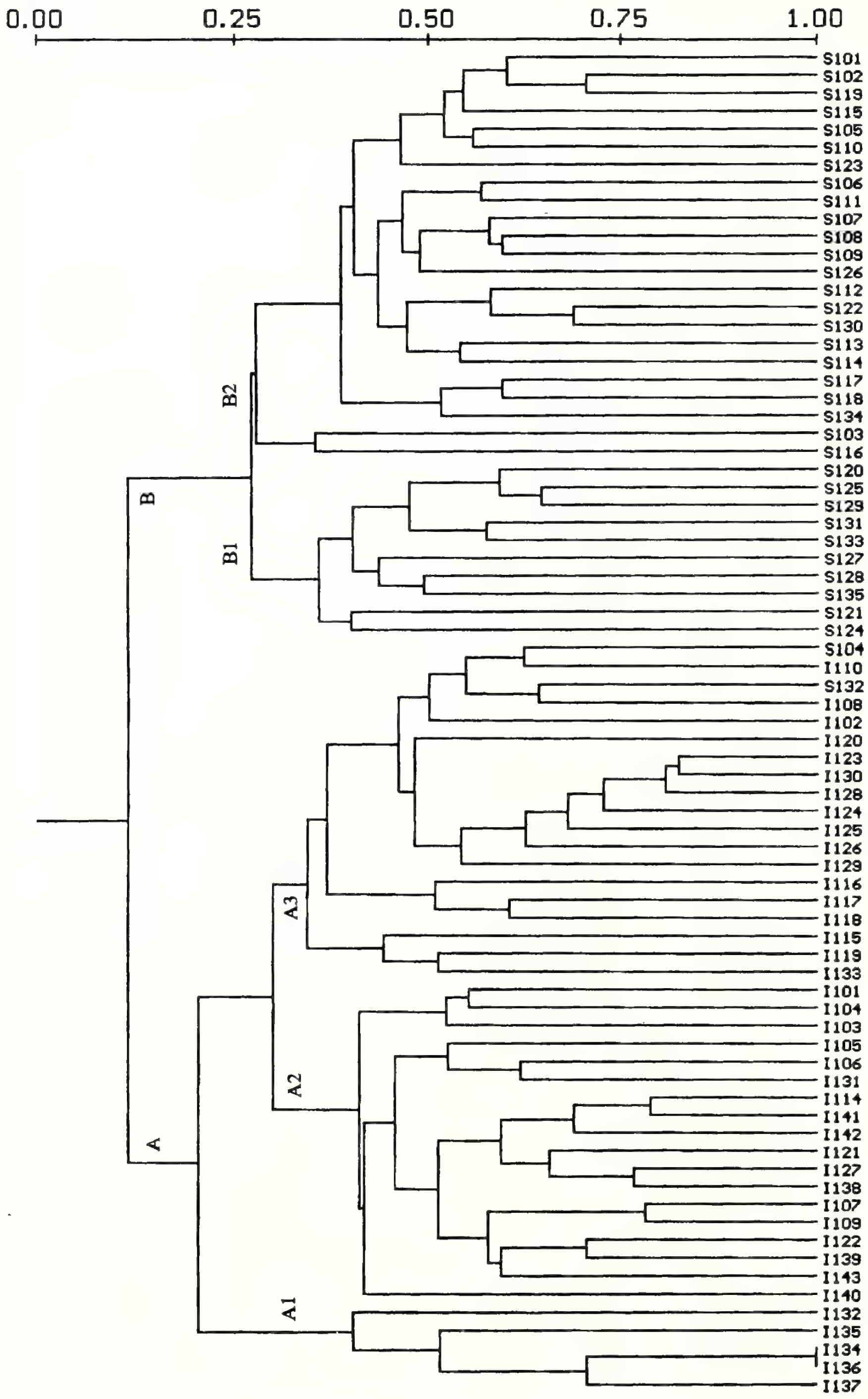

Fig. 4. Clusters of the samples based on the point correlation index and sample of the five main groups.

areas of the ria were deposited there.

After applying the entire series of statistical analyses, a clear coherence can be seen among them, with very similar results obtained from the different methods. A correspondence between the classification analysis (similarity coefficient, Φ) and the ordination analysis (DCA) was found. The results point to the presence of five groups (Fig. 7) characterized as follows:

Subgroup A1 was located in the intertidal zone close to the mouth of the river. It consisted of five stations in which 13 species were found (Table 7). This area had predominantly fine sands (62.43% ± 6.04%) and the lowest

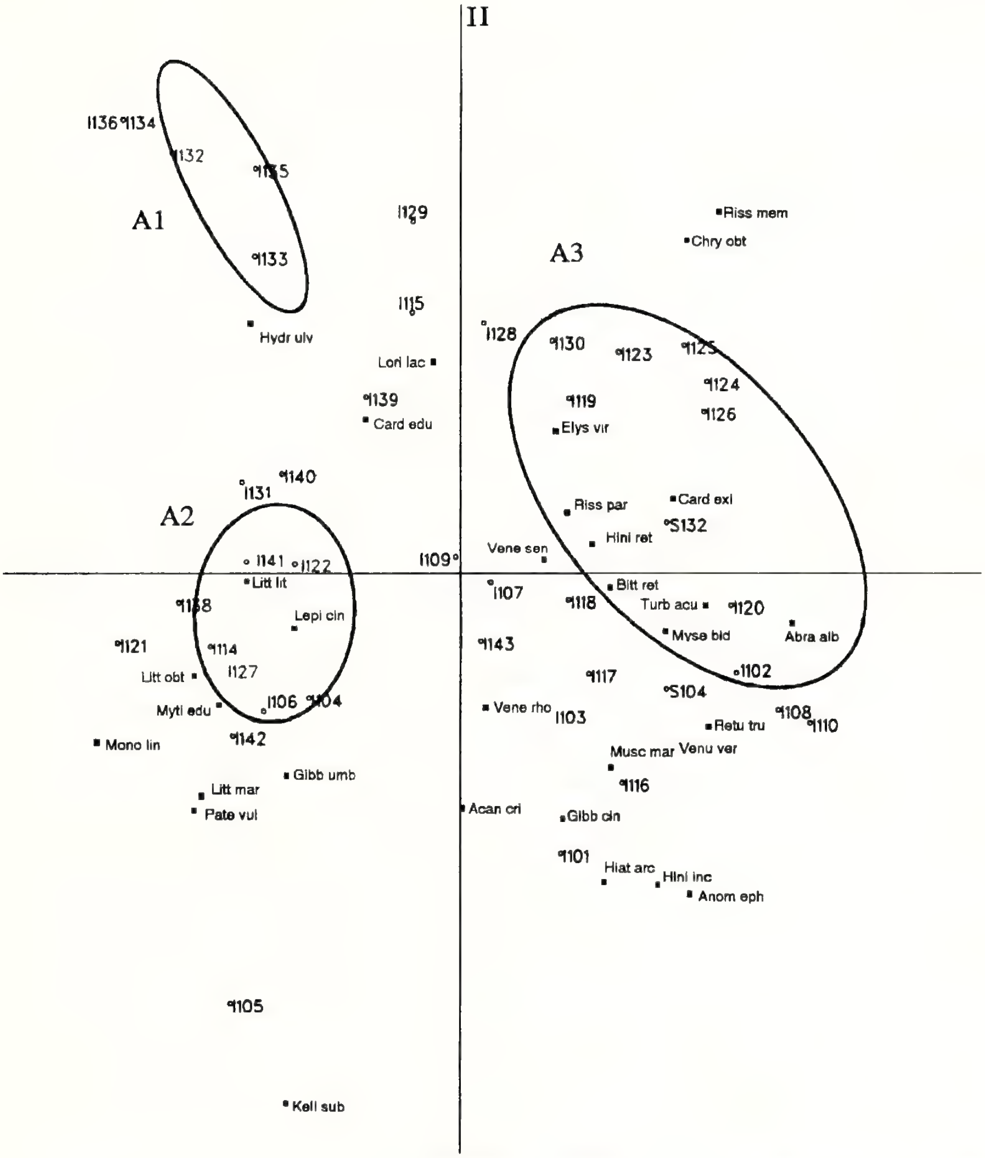

Fig. 5. Stations and species arranged according to axes I and II of the correspondences analysis (DCA) for the intertidal stations, showing the division into three main groups, confirmed by the confidence ellipsis method (Sokal and Rohlf, 1979). Species codes as in Table 7.

mean diversity values (0.53 ± 0.79). Evenness was between 0.91 (station I115) and 0.06 (stations I135, I137), even though this index was undetermined (H' = 0) in two stations by the exclusive presence of the gastropod *Hydrobia ulvae*, which reached very high densities in this subgroup (2,960 ind/m2). There was a small number of species which varied from 5 (station I115) to 1 (stations I134, I135). In this subgroup, the presence of a freshwater bivalve *Pisidium casertanum* was detected. This species' occurrence was probably due to the proximity of the mouth of the river.

This subgroup was represented primarily by *Hydrobia ulvae* (accessory constant; see classification in Table 2), *Scrobicularia plana* (exclusive common), *Cerastoderma edule* (common occasional), and *Rissoa parva* (very common occasional). The FxD value and the dominance indices indicated that *H. ulvae* is most characteristic of this subgroup (Table 7).

Subgroup A2 was located in the intertidal zone on the border of the inlet. It comprised 18 stations in which a total of 29 species were found (Table 7). It had higher mean percentages of gravel and coarse sand than of medium and fine sands. The mean percentage of carbonates (7.22% ± 0.86%) and the mean diversity value (2.77 ± 0.86) were the highest in the area. The evenness values fluctuated from 0.09 (station I139) to 0.43 (station I131) and the number of

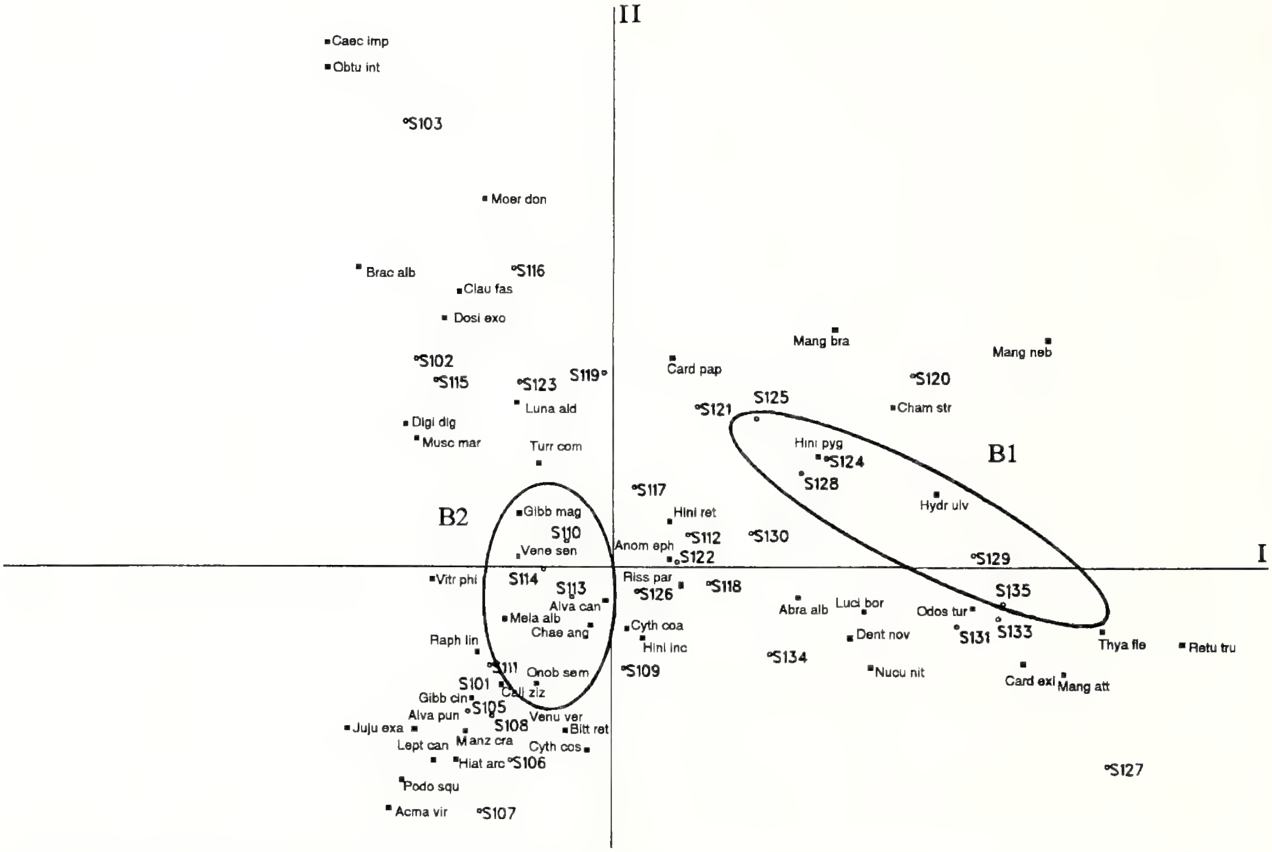

Fig. 6. Stations and species arranged according to axes I and II of the correspondence analysis (DCA) for the subtidal stations, showing the division into two main groups, confirmed by the confidence ellipsis method. Species codes as in Table 8.

species varied from 29 (station I103) to six (station I140).

The characteristic species of this subgroup were *Gibbula umbilicalis, Littorina obtusata* (exclusive constants), *L. littorea* (elective constant), *L. mariae* (very common exclusive), *Lepidochitona cinera* (very common elective), *Mytilus edulis* (very common occasional), *Monodonta lineata*, and *Patella vulgata* (exclusive common). According to the values of FxD and the dominance indices, the most characteristic species of this subgroup were *G. umbilicalis, L. littorea, L. obtusata, L. mariae,* and *L. cinerea* (Table 7).

Subgroup A3 was established in the mid-inner area of the inlet, along with two subtidal stations (S132, S104). It encompassed a total of 17 stations which account for 24 species. This group had high mean percentages of fine sand (45.92% ± 25.60%) and medium sand (21.89% ± 14.63%). The diversity had a lower mean value than in the A2 subgroup (2.35 ± 0.82), evenness varied from 0.09 (station I133) to 0.92 (station I119), and species richness varied from 24 (station I102) to 4 (station I119).

The most noteworthy species were *Parvicardium exiguum* (preferential constant), *Turbonilla acuta, Chrysallida obtusa* (preferential common), *Rissostomia membranacea* (elective common), and *Retusa truncatula* (very common accessory). The FxD index indicated that the most characteristic species of this assemblage were *P. exiguum, T. acuta, C. terebellum*, and *R. membranacea* (Table 7). *Bittium reticulatum* was also present and showed a high dominance and FxD index, making it an important species in the characterization of these bottoms, although it also had a high dominance in subgroups A2 and B2.

Cerastoderma edule, Venerupis senegalensis, and *Hydrobia ulvae* also had high FxD index values in subgroup A2. This shows that there were three important species in the characterization of these intertidal bottoms.

Subgroup B1 was located in the mid-outer subtidal zone of the inlet. It was comprised of ten stations with a total of 31 species. It had relatively high mean percentages of gravel (22.47% ± 1.99%) and fine sand (21.29% ± 20.01%). The percentage of carbonates was lower than in subgroup B2. The mean diversity value was high (3.07 ± 0.62) and evenness fluctuated from 0.94 (station S124) to 0.56 (station S125). The number of species varied from 26 (station S133) to 8 (station S135).

This subgroup was primarily composed of *Thyasira flexuosa* (preferential constant), *Hinia reticulata* (occasion-

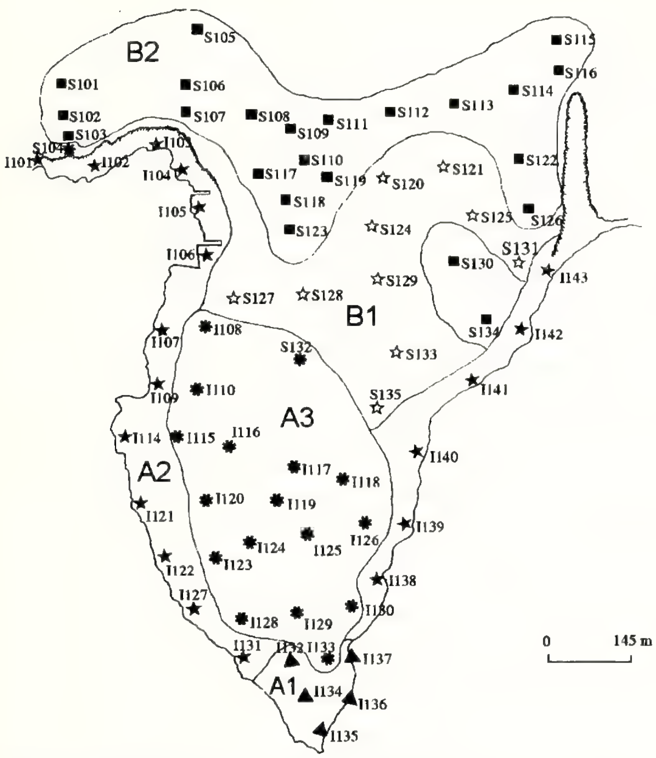

Fig. 7. Distribution of the five groups in the Inlet of Baño. (A1, ▲; A2, ★; A3, ✸; B1, ☆; B2, ■)

al constant), *H. incrassata* (very common accessory), *H. pygmaea* (very common preferential), *Abra alba* (very common accessory), *A. nitida* (exclusive common), *Nucula nitida, Mangelia brachystoma*, and *Odostomia turrita* (preferential common). The highest dominance and FxD values were found for *T. flexuosa, A. nitida*, and *N. nitida* (Table 8).

Subgroup B2 was located in the outer area of the subtidal zone, excluding stations S134 and S130, which were located in the central area. It was comprised of 23 stations encompassing a total of 70 species. It had a heterogeneous granulometric composition, with the lowest mean percentage of fine sand of all the subgroups (7.56% ± 5.01%) and a mean silt-clay value similar to that of subgroup B1 (21.57% ± 12.54%). The mean diversity value (3.44 ± 0.61) was slightly higher than in subgroup B1. Evenness values ranged from 0.37 (station S126) to 0.85 (station S101); species richness values ranged from 45 (station S111) to 18 (station S134).

This subgroup was represented by a great number of constant species which were very common, common, preferential, or elective. According to the values of the dominance and FxD indices, the most characteristic species were: *Lunatia alderi, Manzonia crassa, Gibbula magus, Leptochiton cancellatus, Calyptraea chinensis, Cytharella coarctaca, Raphitoma linearis, Onoba semicostata*, and *Venerupis rhomboides* (Table 8).

In both subgroups B1 and B2, *Hinia reticulata, H. incrassata, Mysella bidentata*, and *Papillicardium papillosum* had high dominance values as well as high FxD indices. It is also important to note the presence of *Rissoa parva*, which appeared as an occasional constant species in subgroups B1 and B2 and attained high values of dominance and FxD. This latter species also appeared as a very common occasional species in three subgroups of intertidal stations (A1, A2, A3). *Venerupis senegalensis* was also widely distributed. This species had a high FxD index, although it was poorly represented in subgroup B1 (occasional not very common), in B2 (occasional constant), and A3 (accessory constant).

DISCUSSION

Species richness and diversity showed a distinct positive correlation with the sort coefficient and a negative correlation with fine sand content. This is logical because the increase in the sort coefficient implies that there is an increase in the diversity of the particles and an increase in heterogeneity, in agreement with Rhoads and Young (1970), Webb (1976), and Lewis (1982), to name but a few, who stated that spatial heterogeneity is conducive to higher diversity. Cornet (1985), however, reported that the nature of the bottom controls community structure, but that the different sediment parameters do not share equal importance. Rather than the dimensions of the sediment particles, it is the variety of the particles that plays the key role. This reflects the microstructural complexity of the habitat in which an increase in sediment variability allows species diversity to rise (Gray, 1974). Moreover, as the grain size decreases, there are restrictions related to the poor microdiversity of the sediment environment, the limited interstitial space, and oxygen diffusion, which justify the negative correlation between diversity and percentage of fine sand.

A negative correlation was found between diversity and the depth parameter, the former diminishing as depth decreased. This can be attributed to the fact that the fauna inhabiting the intertidal zone must tolerate abrupt environmental changes, being faced with problems resulting from changes in salinity, rough conditions on the intertidal floor, danger of desiccation, and extreme temperatures (Kikuchi, 1987).

In the subtidal zone, the highest values for diversity and species richness were found in the stations where the maërl bed was located (*Lithothamnion* and *Phymatolithon*), a bottom composed of shells with a small amount of mud. According to Urgorri *et al.* (1992), these maërl bottoms are

Table 7. Constancy index (%, Cons), fidelity index (%, Fid), dominance (Dom), and fidelity-dominance product (FxD) in subgroups A1, A2 and A3. (*, species present in at least 10% of the stations).

	A1				A2				A3			
Species/ Code	Cons	Fid	Dom	FxD	Cons	Fid	Dom	FxD	Cons	Fid	Dom	FxD
Littorina littorea (Linné, 1758) [Litt lit*]	14	12.38	0.78	173.30	88	77.87	4.51	6,852.56				
Gibbula umbilicalis (da Costa, 1778) [Gibb umb*]					83	94.31	7.42	7,827.73				
Littorina obtusata (Linné, 1758) [Litt obt*]					77	100.00	13.37	7,700.00				
Cerastoderma edule (Linné, 1758) [Card edu*]	42	23.72	0.55	996.20	83	46.89	1.75	3,891.87	52	29.37	2.34	1,527.24
Hydrobia ulvae (Pennant, 1777) [Hydr ulv*]	100	39.37	97.40	3,937.00	88	34.64	19.54	3,048.32	52	20.47	25.27	1,064.44
Venerupis senegalensis (Gmelin, 1791) [Vene sen]					77	29.27	3.60	2,253.79	88	33.46	3.15	2,944.48
Littorina mariae Sacchi & Rastelli, 1966 [Litt mar*]					55	100.00	15.20	5,500.00				
Lepidochitona cinerea (Linné, 1767) [Lepi cin*]					72	72.00	1.36	5,184.00				
Mytilus edulis Linné, 1758 [Myti edu*]	14	8.80	0.27	123.20	72	42.58	7.45	3,260.16	23	14.46	1.08	332.58
Rissoa parva (da Costa, 1778) [Riss par]	57	14.21	0.32	809.90	72	17.95	12.32	1,292.40	94	23.44	28.45	2,203.36
Bittium reticulatum (da Costa, 1778) [Bitt ret]					66	27.04	2.55	1,784.64	70	28.68	5.38	2,007.60
Scrobicularia plana (da Costa, 1778) [Scro pla]	28	100.00	0.18	2,800.00								
Patella vulgata Linné, 1758 [Pate vul*]					50	100.00	0.61	5,000.00				
Monodonta lineata (da Costa, 1778) [Mono lin*]					38	100.00	0.48	3,800.00				
Acanthochitona crinita (Pennant, 1777) [Acan cri*]					33	66.00	0.25	2,178.00				
Elysia viridis (Montagu, 1804) [Elys vir*]	14	14.89	0.09	208.40	33	35.10	0.23	1,155.00	47	50.00	1.11	2,350.00
Chrysallida obtusa (Brown, 1827) [Chry obt]									41	65.07	0.02	2,667.87
Gibbula cineraria (Linné, 1758) [Gibb cin*]					44	28.02	1.03	1,232.88	35	22.29	0.51	780.15
Turbonilla acuta (Donovan, 1804) [Turb acu*]					27	32.92	0.48	888.84	47	63.51	1.20	2,984.97
Mysella bidentata (Montagu, 1803) [Myse bid]	14	4.44	0.13	62.16	50	15.87	2.57	793.51	70	22.22	7.39	1,555.40
Hinia reticulata (Linné, 1758) [Hini ret]	14	4.00	0.04	56.00	50	14.45	3.89	722.50	82	23.69	2.40	1,942.58
Retusa truncatula (Brugière, 1792) [Retu tru*]					27	19.00	1.72	513.00	52	36.61	2.06	1,903.72
Abra alba (Wood, 1802) [Abra alb*]									52	29.71	1.11	1,544.92
Parvicardium exiguum (Gmelin, 1791) [Card exi*]					27	17.53	1.83	468.45	94	61.03	2.46	5,736.82
Venus verrucosa Linné, 1758 [Venu ver*]									29	15.42	0.17	447.18
Pisidium casertanum (Poli, 1791) [Pisi cas]	14	100.00	0.04	1,400.00								
Chrysallida terebellum (Philippi, 1844) [Chry ter]	14	46.60	0.04	652.40								
Cerithiopsis tubercularis (Montagu, 1803) [Ceri tub]					16	100.00	0.69	1,600.00				
Kellia suborbicularis (Montagu, 1803) [Kell tub]					22	62.85	1.57	1,382.70				
Dosinia exoleta (Linné, 1758) [Dosi exo]	14	12.28	0.04	171.90								
Brachystomia albella (Lovén, 1843) [Brac alb]					22	33.84	0.23	854.48				
Alvania semistriata/ (Montagu, 1808) [Alva sem]					16	42.10	0.72	673.60				
Rissostomia membranacea (Adams, 1800) [Riss mem]					16	31.37	0.10	501.92	35	68.62	8.62	2,401.70
Moerella donacina (Linné, 1758) [Moer don]									17	23.94	0.40	406.98
Onoba semicostata (Montagu, 1803) [Onob sem]									17	13.07	0.14	222.19
Chamelea striatula (da Costa, 1778) [Cham str]									17	22.07	0.08	375.19
Hinia incrassata (Ström, 1768) [Hini inc*]									23	12.50	0.31	287.50
Anomia ephippium Linné, 1758 [Anom eph*]									23	24.73	0.94	569.25
Loripes lacteus (Linné, 1758) [Lori lac*]	14	25.45	0.13	356.30	16	29.09	0.07	465.44				
Musculus marmoratus (Cantraine, 1835) [Musc mar*]					16	21.91	0.54	350.56	23	31.50	0.28	724.50
Hiatella arctica (Linné, 1767) [Hiat arc*]					16	15.84	0.79	253.44	29	28.71	0.25	832.59

stable substrata that provide a good shelter for small mollusks, because they have small spaces which favor the settlement of species typical of hard substrata. A large number of these individuals inhabit these bottoms, settling on branches of seaweed. There are also species typical of muddy bottoms which are found in the lower area or in contact with the sandy substratum.

Relatively high values of diversity and species richness were found in the intertidal zone in stations with *Zostera noltii*. The seagrass gives rise to a complex habitat with richer fauna (Peterson *et al.*, 1984), either because it stabilizes the sediment or because it shelters certain species, creating more complex microhabitats which are non-existent in unprotected zones.

The minimum values of species richness and diversity were found in the intertidal stations in proximity to the mouth of the river, owing to the almost exclusive dominance of *Hydrobia ulvae*, which reached high densities. This gastropod enjoys a broad range of food sources, which enables it to thrive in highly varied environments, feeding on organic remains, fecal pellets, or sediment particles, behaving as a detritivore (Jacobs *et al.*, 1983) or an herbivore consuming microalgae obtained by grazing (Muus, 1967). According to Wolff (1973), the high densities of this gastropod are linked to intertidal areas having little water movement and high sediment stability.

Generally speaking, the diversity values observed in the inlet were high. When compared with the results of other benthic studies on mollusks, our values were higher than those found in the Ría de Ares-Betanzos (Troncoso *et al.*, 1993), the Gulf of Gascogne (Cornet, 1985, 1986), and in Raunfjorden (Tunberg, 1981), and were similar to those reported on the southern banks of the Lake of Tunis (Zaouali, 1974, 1981).

Two communities were described in the area under study. However, the sediment heterogeneity in this zone causes the occurrence of phenomena such as facies and transitions. In the subtidal zone (group B), the community of *"Syndosmia"* (Thorson, 1957) was encountered, structured in facies (B1, B2). This community, settled in the subtidal zone, has been found in several areas: in the Ría de Arousa (Cadée, 1968; Mora, 1980), in the Bay of Santander (Lastra *et al.*, 1990), in populations of the western English Channel (Cabioch, 1968), off the coast of Scotland (Pearson, 1970), in the Rade de Brest (Hily, 1976), and in the Gulf of Normandy-Brittany (Retier, 1979; Gentil, 1982).

The subgroup B1 represents the facies of *Thyasira flexuosa-Nucula nitida*, which is typical of bottoms having fine sediment particles and a high silt-clay ratio. Glémarec (1964) described this facies in the Gulf of Morbihan as the "*Nucula turgida* type C" facies with *N. turgida, Abra nitida*, and other species such as *T. flexuosa*. Subgroup B2 represents a facies in transition toward the community of *Clausinella fasciata*, having bottoms with larger-sized particles than in B1, but with a relatively high silt-clay ratio, which causes it to be characterized by species both typical of the community of *"Abra alba"* as well as by species typical of the community of *"C. fasciata"* (*Dosinia exoleta, Pododesmus squamula, Gouldia minima, C. fasciata, Venerupis rhomboides*, and *Moerella donacina*). Also found within this facies (S101, S102, S107, S108) is a maërl bottom, with the above-mentioned typical species present. The accumulation of *Lithothamnion* and *Pymatholiton* on the sediment creates a particular algal epifauna and endofauna, which is why many authors have described the facies as belonging to the community of *Clausinella fasciata* (*e. g.*, Glémarec, 1965; Cabioch, 1968; Keegan, 1974; Mora, 1980).

On the intertidal bottoms (group A) a small community of *"Macoma"* (Thorson, 1957) was encountered structured in facies (A1, A2, A3). According to Thorson (1957) the small community of *"Macoma"* (community of *Cerastoderma edule-Scrobicularia plana*) is a variety of the community of *M. balthica*. Although this community has been cited from higher latitudes, in its purest sense, it does not extend beyond the English Channel, where species giving rise to the variety *C. edule-S. plana* become more frequent. In the zone under study, this community was found on bottoms of fine sand. A clear dominance of *Hydrobia ulvae* was seen and high dominance values were also cited in other areas of the European coast (Denis, 1983).

Cadée (1968) and Mora (1980) found similar communities on Galician coasts. In the Ría de Arousa they described a community of *"Cerastoderma edule-Scrobicularia plana"* in the polyhaline zone. Viéitez (1976) described the same community on the beach of Meira (Ría de Vigo), which is known to settle on bottoms of fine sand with mean values of organic matter of 0.85%. Penas and González (1983) reported the presence of this community in the Ría de Arousa; Planas (1986) studied this community in the Inlet of Lourizán (Ría de Pontevedra) on bottoms primarily composed of a coarse fraction. Junoy and Viéitez (1990) found this community in the Ría de Foz and it was distinguished by favoring bottoms having silt-clay percentages of over 5% and relatively high organic matter content, where some species which were highly abundant, such as *Hydrobia ulvae*, are typical of estuaries having organic pollution (Viéitez, 1981; Penas and González, 1983; Planas and Mora, 1984b; Planas *et al.*, 1984).

Within this community, subgroup A1 comprises a facies dominated almost exclusively by *Hydrobia ulvae*, and to a lesser extent by *Scrobicularia plana. H. ulvae*, which reached the highest macrofaunal abundances in the study area, forms a typical estuarine population that tolerates eurythermal and euryhaline conditions. Subgroup A2

Table 8. Constancy index (%, Cons), fidelity index (%, Fid), dominance (Dom), and fidelity-dominance product (FxD) in subgroups B1 and B2. (*, species present in at least 10% of the stations).

	B1				B2			
Species/ Code	Cons	Fid	Dom	FxD	Cons	Fid	Dom	FxD
Lunatia alderi (Forbes, 1838) [Luna ald*]					78	88.63	0.39	6,913.14
Cytharella coarctata (Forbes, 1840) [Cyth coa*]	40	31.74	1.50	1,269.60	86	65.66	1.80	5,646.76
Turritella communis Risso, 1826 [Turr com]	50	36.76	0.46	1,838.00	86	63.00	2.36	5,418.00
Calyptraea chinensis (Linné, 1758) [Caly chi*]	60	41.00	1.04	2,460.00	86	58.90	2.84	5,065.40
Onoba semicostata (Montagu, 1803) [Onob sem*]	30	23.00	0.46	690.00	78	60.00	10.04	4,680.00
Papillicardium papillosum (Poli, 1795) [Card pap*]	80	47.90	6.82	3,832.00	82	49.10	3.55	4,075.40
Hinia incrassata (Ström, 1768) [Hini inc*]	70	38.00	6.01	2,660.00	86	46.70	5.35	4,016.50
Cerastoderma edule (Linné, 1758) [Card edu]					80	49.77	1.67	3,981.87
Gibbula cineraria (Linné, 1758) [Gibb cin*]					78	49.68	2.87	3,875.00
Venus verrucosa Linné, 1758 [Venu ver*]					78	45.34	1.23	3,536.52
Hinia reticulata (Linné, 1758) [Hini ret]	100	28.90	10.98	2,890.00	100	28.90	4.66	2,890.00
Mysella bidentata (Montagu, 1803) [Myse bid]	90	28.57	23.93	2,571.30	91	28.80	24.69	2,628.08
Bittium reticulatum (da Costa, 1778) [Bitt ret]	30	12.29	0.46	368.70	78	32.00	3.00	2,496.00
Venerupis senegalensis (Gmelin, 1791) [Vene sen]	20	7.60	0.23	152.00	78	29.65	5.41	2,312.70
Rissoa parva (da Costa, 1778) [Riss par]	100	24.93	4.39	2,493.00	78	19.45	3.47	1,517.10
Manzonia crassa (Kanmacher, 1798) [Manz cra*]					69	93.24	2.06	6,433.56
Gibbula magus (Linné, 1758) [Gibb mag*]					60	100.00	0.43	6,000.00
Leptochiton cancellatus (Sowerby, 1840) [Lept can*]					60	92.30	0.75	5,538.00
Venerupis rhomboides (Pennant, 1777) [Vene rho*]	20	20.83	0.57	416.60	65	67.70	0.84	4,400.50
Alvania punctura (Montagu, 1803) [Alva pun*]					52	83.87	1.56	4,361.24
Clausinella fasciata (da Costa, 1778) [Clau fas*]					52	82.53	0.61	4,291.56
Raphitoma lineraris (Montagu, 1803) [Raph lin*]					52	100.00	0.26	5,200.00
Dosinia exoleta (Linné, 1758) [Dosi exo*]	20	17.54	0.46	350.80	69	60.52	0.90	4,175.80
Hiatella arctica (Linné, 1767) [Hiat arc*]					56	55.40	0.74	3,104.64
Gouldia minima (Montagu, 1803) [Goul min*]	40	43.47	1.15	1,738.80	52	56.52	0.64	2,939.04
Hinia pygmaea (Lamarck, 1822) [Hini pyg*]	70	57.37	1.73	2,660.00	52	42.60	0.54	2,215.20
Abra alba (Wood, 1802) [Abra alb*]	60	34.28	4.50	2,056.80	52	29.71	1.36	1,544.92
Abra nitida (Müller, 1776) [Abra nit]	40	100.00	2.42	4,000.00				
Mangelia nebula (Montagu, 1803) [Mang neb*]	30	78.94	0.46	2,368.20				
Acmaea virginea (Müller, 1776) [Acma vir*]					34	100.00	0.29	3,400.00
Vitreolina philippii (Rayneval & Ponzi, 1854) [Vitr phi*]					26	100.00	0.08	2,600.00
Melanella alba (da Costa, 1778) [Mela alb*]					47	82.45	0.34	3,875.15
Pododesmus squamula (Linné, 1758) [Podo squ*]					39	88.63	5.52	3,456.57
Digitaria digitaria (Linné, 1758) [Digi dig*]					39	79.59	0.19	3,104.01
Brachystomia albella (LovÈn, 1843) [Brac alb*]					43	68.25	0.08	2,600.00
Jujubinus exasperatus (Pennant, 1777) [Juju exa*]					30	75.00	0.44	2,250.00
Caecum imperforatum (Kanmacher, 1798) [Caec imp*]					26	83.80	0.09	2,178.80
Obtusella intersecta (Wood, 1856) [Obtu int*]					26	72.00	0.01	1,760.20
Moerella donacina (Linné, 1758) [Moer don*]					39	54.92	0.19	2,141.88
Myrtea spinifera (Montagu, 1803) [Myrt spi]	20	20.00	0.23	400.00				
Lucinoma borealis (Linné, 1767) [Luci bor*]	20	31.25	0.23	625.00	34	53.12	0.09	1,806.08
Anomia ephippium Linné, 1758 [Anom eph*]	20	21.50	1.61	430.00	39	41.93	3.04	1,635.27
Mangelia brachystoma (Philippi, 1844) [Mang bra*]	40	57.14	0.92	2,285.60	30	42.87	0.11	1,286.10
Chamelea striatula (da Costa, 1778) [Cham str*]	30	38.96	1.15	1,168.80	30	38.96	0.21	1,168.80
Nucula nitida Sowerby, 1833 [Nucu nit*]	50	65.78	1.15	3,289.00	26	34.21	0.08	889.46
Musculus marmoratus (Cantraine, 1835) [Musc mar*]					34	46.57	0.32	565.80
Thyasira flexuosa (Montagu, 1803) [Thya fle*]	100	64.93	15.72	6,493.00	43	27.92	0.40	1,200.99
Mytilus edulis Linné, 1758 [Myti edu*]	20	12.57	0.23	975.60	30	18.86	0.23	889.46
Gibbula tumida (Montagu, 1803) [Gibb tum]					17	100.00	0.13	1,700.00
Alvania beani (Thorpe, 1844) [Alva bea]					17	100.0	0.12	1,700.00
Alvania cancellata (da Costa, 1778) [Alva can*]					17	100.00	0.09	1,700.00
Caecum glabrum (Montagu, 1803) [Caec gla]					17	100.00	0.05	1,700.00
Haedropleura septangularis (Montagu, 1803) [Haed sep]					17	100.00	0.03	1,700.00
Odostomia conspicua Alder, 1850 [Odos con]					13	100.00	0.06	1,300.00
Monia patelliformis (Linné, 1767) [Moni pat]					13	100.00	0.04	1,300.00
Brachystomia eulimoides (Hanley, 1844) [Brac eul]					13	100.00	0.03	1,300.00

(continued)

Table 8. Continued

	B1				B2			
Species/ Code	Cons	Fid	Dom	FxD	Cons	Fid	Dom	FxD
Callochiton achatinus (Montagu, 1803) [Call ach]					13	100.00	0.02	1,300.00
Calliostoma zizyphinum (Linné, 1758) [Call ziz*]					21	67.00	0.07	1,407.00
Dentalium novencostatum Lamarck, 1838 [Dent nov*]					21	67.00	0.06	1,407.00
Diodora graeca (Linné, 1758) [Diod gra]					13	100.00	0.03	1,300.00
Chrysallida indistincta (Montagu, 1808) [Chry ind]					13	72.20	0.03	938.60
Chaetopleura angulata (Splenger, 1797) [Chae ang*]					21	58.00	0.06	1,218.00
Cytharella costata (Donovan, 1803) [Cyth cos*]	20	48.78	0.23	975.60	21	51.21	0.13	1,075.41
Mangelia attenuata (Montagu, 1803) [Mang att*]	20	60.60	0.46	1,212.00	13	56.52	0.02	734.76
Alvania semistriata (Montagu, 1808) [Alva sem]					17	44.73	0.78	760.41
Acanthochitona crinita (Pennant, 1777) [Acan cri]					17	34.00	0.05	578.00
Kellia suborbicularis (Montagu, 1803) [Kell sub]					13	37.14	0.03	482.82
Odostomia turrita Hanley, 1844 [Odos tur*]	30	57.69	0.92	1,730.70	17	32.69	0.11	555.73
Chrysallida obtusa (Brown, 1827) [Chry obt]					17	29.63	0.05	452.71
Ostrea edulis Linné, 1758 [Ostr edu]					13	28.88	0.02	375.44
Lepidochitona cinerea (Linné, 1767) [Lepi cin]					17	17.00	0.12	289.00
Retusa truncatula (Bruguière, 1792) [Retu tru*]	50	35.21	1.04	1,760.50	13	9.10	0.03	118.30
Parvicardium exiguum (Gmelin, 1791) [Card exi*]	20	12.98	0.23	152.00	13	8.40	0.08	109.20
Hydrobia ulvae (Pennant, 1777) [Hydr ulv*]	20	7.50	0.21	150.00	13		0.04	63.89

constitutes another facies within the community of *"Macoma,"* settled on bottoms having coarser sediment with a low silt-clay content. Cadée (1968) found a similar fauna in the shallow border area of the Ría de Arousa, but this author integrated it into a community of *"Tellina"* (Thorson, 1957). However, according to Cadée (1968), the resemblance is not exact, because the genera characteristic of this community are not found very often, but rather the area, in addition to the dominant presence of the typical species of this community, has other species characteristic of fine substrata, where there are stones to which algae become attached thus providing food for *Littorina littorea*, *L. obtusata*, *Gibbula umbilicalis*, etc. The fauna belonging to this facies can be associated with the intertidal zone of *Laminaria* reported by Jeffreys (1863). Subgroup A3 consists of a facies located in an area with a relatively high silt-clay content and where *Zostera noltii* settles. The biocenotic classification of these bottoms is difficult, because, as explained by Pérès (1958), the community of *Zostera* is a complex community where three compartments can be distinguished: the sessile or sedentary population of the leaves, the sediment population, and the vagile population living in the shade of the leaves. Currás and Mora (1991) stated that these bottoms should be associated with a particular biocenosis with endofauna typical of nearby populations and lacking a vegetative covering. Petersen (1918) reported that the nature of the epifauna of *Zostera* is determined by the type of animal community where it settles. Pérès and Picard (1964) included the facies of *Z. noltii* within the biocenosis of superficial silty sands in the calm zones. Muus (1967) studied the bottoms of *Zostera* and considered the fauna as an animal community corresponding to the community of *"Macoma."* The results concur with the opinion of Currás and Mora (1991), because upon analyzing the most significant species in this subgroup, it can be seen that it is a facies of the small community of *Macoma*, with an endofauna characteristic of this community (*Loripes lacteus, Cerastoderma edule*, and *Venerupis senegalensis*) and an epifauna of euryhaline species such as *Hydrobia ulvae, Bittium reticulatum*, and *Rissostomia membranacea*.

Another important aspect is the determination of the environmental factors that affect the settlement of the communities. Penas and González (1983), Currás and Mora (1991), and Bachelet and Dauvin (1993), to name but a few, considered that the proportion of the different granulometric classes, silt-clay content, organic matter, and the presence or absence of vegetative cover are the determining factors in the structuring of benthic communities. Our data coincide with the results of the above-mentioned authors, because in the subtidal zone the physico-chemical factors that will be more important in faunal distribution are the sediment gradient and the organic matter content. This is to be expected because this is a narrow bathymetric range, the variation of other physical parameters (temperature, salinity), as pointed out earlier by Jones (1950: 293), will be small, and the decisive factor will be the granulometry of the bottom which is a consequence of the peculiar hydrodynamic regime in the area.

In addition to the granulometric nature of the substratum in the intertidal zone, which is one of the most important factors in the distribution of the species, depth plays an important role in the distribution of the species because the higher intertidal levels present rougher conditions for aquatic organisms. The almost total absence of water during the emersion period prevents them from using this environment to be active and to feed during this period, during which time there is an increased danger of desiccation and freezing. This is why the species on the higher levels have morphological, physiological, ecological, or ethological adaptations that are different from species living at lower levels. The importance of tidal level and zoning, because this implies different types of adaptations, has also been discussed by several authors (Wolff, 1973; Raffaelli and Boyle, 1986; Viéitez and Baz, 1988; Penas and González, 1983; Eleftheriou and Robertson, 1988; Junoy and Viéitez, 1990).

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Predatory gastropod traces: a comparison of verified shallow-water and presumed deep-sea boreholes

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Abstract: The present study was based on a light microscopic examination of gastropod-type boreholes in shells of deep-sea mollusks dredged by the *Galathea* Expedition at stations off of southern Africa, east of India, in the Indonesian region, and off of southern Australia; the *Ingolf* Expedition in the North Atlantic; and the *Gosnold* survey off of the eastern coast of the United States. The morphology of these holes is closely similar to that of boreholes drilled by shallow-water naticodean and muricodean gastropods. This coincidence in form supports the conclusion that these deep-sea holes were also bored by gastropods. Boreholes, in shells dredged as deep as 2690 m, ranged in external diameter from 0.2-3.1 mm, were round, smooth-walled, and significantly smaller than those drilled by shallow-water gastropods. The presence of gastropod boreholes in prey shells from the deep sea is not surprising, as species of Naticidae, Muricidae, and Trophonidae, the first more commonly, have been dredged from several oceanic stations, naticids from as deep as 6860 m. In the present study, muricid-type boreholes were 1/7 as common as naticid-type holes, probably a reflection of the lower density of muricid species and the soft surface of much of the ocean floor, which, at least in shallow water, tends to inhibit muricid reproduction.

Key words: gastropods, predators, shell borers, boreholes, deep-sea

No one has yet observed living deep-sea predatory gastropods in the act of drilling holes in living deep-sea molluscan prey. Thus the few holes that have been reported as deep-sea boreholes (for example, Boone and Carriker, 1960; Knudsen, 1970), have been so named because of their apparent likeness to those excavated under laboratory conditions by shallow-water predatory gastropods. The identity of holes of many species of shallow-water boring gastropods has been verified in the laboratory and in the field (*e. g.*, Wiltse, 1980; Carriker, 1981; Ansell and Morton, 1987; Vermeij *et al.*, 1989; Ponder and Taylor, 1992; Peterson and Black, 1995). Because all shallow-water boring predatory gastropods examined to date have been shown to possess an accessory boring organ (ABO) (Carriker, 1981; Kabat, 1990), suspected deep-sea boring gastropods could be confirmed as borers, anatomically and histologically, by the presence of an ABO. Harasewych (1984) has thus identified a bathyal trophonid as a borer.

Although taxonomically identified naticids, muricids, and trophonids have been dredged from the deep sea, and occasional figures of boreholes in molluscan valves, presumably drilled by species in these and possibly other taxa, have been published, no systematic study of the comparative morphology of gastropod-type boreholes from the deep sea has been undertaken.

The purpose of the present study was to compare the gross morphological and light microscopical structure of boreholes of shallow-water naticids and muricids with that of presumed boreholes in molluscan shells taken at different depths in deeper regions of the oceans. The comparison was made to ascertain the degree, if any, of the resemblance of the two groups of perforations. Molluscan shells were dredged by expeditions of the Danish *Ingolf* (1895 and 1896), the Danish *Galathea* (1950-1952), and the United States *Gosnold* (1963-1964).

RECENT SHALLOW-WATER BORERS

Shell dissolution and penetration have been reviewed by Carriker *et al.* (1969) in many species of prey invertebrates. In the Mollusca, shell penetration occurs among the Bivalvia (burrowing for shelter), Gastropoda, and Cephalopoda (drilling to obtain food). Among the Gastropoda, species of boring snails are present in the families Capulidae, Naticidae, Cassidae, Tonnidae, Ranellidae, Muricidae, Marginellidae, Rapanidae, Trophonidae, Buccinidae, and Vayssiereidae; among the Cephalopoda, borers exist in at least the family Octopodidae (Orr, 1962; Wodinsky, 1969; Young, 1969; Carriker, 1981; Kabat, 1990; Ponder and Taylor, 1992; Egorov, 1993; Gordillo, 1994; Peterson and Black, 1995).

Boreholes made by snails in gastropod families (less the Cassidae) are characteristically gastropod boreholes; that is, they possess smooth walls, bevelled outer edges, decreasing diameter with depth, and are generally circular (Carriker and Yochelson, 1968; Kabat, 1990). Boreholes of species of Cassidae have smooth exterior margins but strongly jagged inner margins (Abbott, 1968; Day, 1969). Those of the Octopodidae are highly irregular in size, shape and angle of penetration (Wodinsky, 1969). All species of borers in the Apogastropoda (less the Capulidae) penetrate the shell of prey to rasp out the flesh within; whereas the Capulidae take food from the food-gathering tract through a borehole over the tract (Orr, 1962).

RECENT SHALLOW-WATER NATICOIDEAN AND MURICOIDEAN BOREHOLES

Naticoidean gastropods commonly live and crawl through clean to slightly muddy-sandy sediment, avoiding sticky, more compacted argillaceous sediments. When they locate an infaunal mollusk (bivalve, scaphopod, or another gastropod), they burrow to its level in the sediment and drill into it there. They possess an exceptionally large fleshy foot that facilitates movement through the sediment, and with it tightly grip their prey. If the prey is smaller than the snail, the snail can completely envelope the prey with its broad foot (Kabat, 1990).

Muricoidean snails, on the other hand, generally dwell on the surface of firm to hard substrata, and prey mostly upon epifauna (bysate bivalves, such as oysters and barnacles). The size of the foot, relative to that of the shell, is very small - quite in contrast to the relatively large foot of naticoideans. Consequently, when a muricid mounts a prey to perforate it, its foot clings tightly to the surface of the prey. Most muricid species, such as *Urosalpinx cinerea* (Say, 1822) drill principally through one of the valves of prey (side drilling); whereas others, like *Muricanthus fulvescens* (Sowerby, 1834), bore at the line of contact of the two valves opposite the hinge (edge drilling), the boreholes thus assuming a long, oval outline (Carriker, 1961). Edge drilling is also done by some polinicine naticids (Vermeij *et al.*, 1989).

CHARACTERISTICS OF SHALLOW-WATER GASTROPOD BOREHOLES

Gastropod boreholes, although varying in size with the stage in the life history of individuals in a single predatory species, are characterized by a number of identifiable features. These are described here briefly as background for the following discussion of the morphology of the deep-sea boreholes.

Macroscopically, the exterior rim or margin and the interior wall of the borehole appear smooth; under magnification, radular teeth marks are visible. The inner edge of the hole (next to the soft tissues of the prey) can be sharp-lipped, smooth, or quite jagged; whereas, the exterior rim ranges from scarcely bevelled to deeply countersunk. The exterior rim is often discolored by action of the accessory boring organ secretion (Carriker, 1981). In section parallel to the exterior surface of the shell, holes can be circular, crescent-shaped, heart-shaped, or highly irregular, but most commonly are circular, a result of the back-and-forth turning of the radula-supporting odontophore on its long axis. The depth axis of muricid boreholes is generally perpendicular to the external shell surface because of the radial symmetry of the extended muricid accessory boring organ and the perpendicular position of the longitudinal axis of its stalk to the ventral surface of the foot. Perpendicularity is also characteristic of some naticid boreholes, especially those drilled in relatively smooth-shelled bivalves. This results from the position of the accessory boring organ on the ventral tip of the naticid proboscis. Symmetry of muricid boreholes can be warped by ornamentation, growth irregularities, and differences in hardness of prey shell, resulting in oval or smoothly angular holes. Exaggerated external shell sculpture such as costae, concentric ridges, imbrications, flutings, and the like, can be reflected on the side of the borehole (Carriker and Yochelson, 1968; Kabat, 1990).

In prey shell composed of homogeneous material (Carriker, 1996), boreholes are uniformly symmetrical. However, the presence of layers of shell material of different mineral composition or hardness can result in irregularities in the diameter of the borehole (Carriker and Yochelson, 1968).

Because of the proportionally small size of their accessory boring organ and stalk, small drilling gastropods successfully penetrate only small, thin-shelled prey (Carriker, 1957; Carriker and Van Zandt, 1972). The diameter of the borehole (at the external surface of the shell) generally indicates the size of the predator; but because small adult snails also excavate holes, the size of hole does not necessarily indicate the age of the borer. Although it is not always possible to distinguish holes bored by naticids from those by muricids, the two types are quite distinctive in a populational sense: typical naticid boreholes are broadly parabolic, whether excavated in thick or thin prey shells, whereas muricid holes appear parabolic in thin prey shells, but in thicker shells the borehole assumes the characteristic cylindrical or nearly cylindrical form with a minor bevel on the exterior margin.

MATERIALS AND METHODS

Molluscan shells for the examination of holes attributed to deep-sea gastropods, were kindly loaned by Dr. Jørgen Knudsen, Zoological Museum, University of Copenhagen, Denmark. These shells were dredged during

(a) the Danish Deep-sea Expedition Round the World, 1950-1952, from the *Galathea* (Brunn, 1957b), and (b) the Danish North Atlantic voyage, 1895 and 1896, from the *Ingolf* (Jensen, 1912). A sample of shells was also generously made available by Mr. Roger B. Theroux, Northeast Fisheries Science Center, National Marine Fisheries Service, NOAA, Woods Hole, Massachusetts, United States. They were collected from the *Gosnold* during the United States Geological Survey Continental Margin Program in 1963-1964 (Emery and Schlee, 1963; Wigley and Theroux, 1981), off of the Middle Atlantic Bight region of the western North Atlantic. Specimens studied were loaned from the Specimen Reference Collection of the Northeast Fisheries Science Center, and are now housed in the National Museum of Natural History, Smithsonian Institution, Washington, D. C.

The *Galathea* shell specimens were collected at stations off of southern Africa, east of India, east of the Philippines, around Bali, and off of southern Australia, at depths ranging from 435-2690 m. Dr. Knudsen selected shells with holes from dredgings deeper than 400 m. Twelve stations were included, between 400 m and the maximum depth of 10,210 m (Bruun, 1957b) (Table 1).

Dr. Knudsen also selected *Ingolf* bivalves [all *Hyalopecten frigidus* (Jensen, 1912)] with holes in specimens collected at stations between the Faroes, Iceland, and

Table 1. Bathymetric distribution of gastropod boreholes in valves of deep-sea mollusks collected by the *Galathea*.

Sta.	Depth (m)	Coordinates	Geographic region	No. boreholes	Borehole diameter (mm)	Mollusk bored	Type borehole
99	2690	8°40'S 11°10'E	off Angola, South Africa	3	1.1, 2.0, 2.2	*Cuspidaria* sp.	muricid
137	535-570	20°04'S 11°56'E	off South Africa	1	1.9	*Venericardia* sp.	muricid
192	3430	32°00'S 32°41'E	off Durban, South Africa	2	0.8, 1.5	*Janthina*-like?	?
302	1190	19°42'N 86°48'E	Bay of Bengal	1	1.1	*Syndosmya longicallis* (Scacchi, 1834)	muricid
423	810	10°27'N 124°18"E	east of Cebu, Philippines	1	1.2	venerid-like	naticid
436	710	10°12'N 124°14'E	east of Cebu, Philippines	1	1.2	*Limopsis* sp.	muricid
436	710	10°12'N 124°14'E	east of Cebu, Philippines	2	2.5, 2.5	gastropod	naticid
436	710	10°12'N 124°14"E	east of Cebu, Philippines	2	2.8, 2.9	*Macoma*-like	naticid
478	600	8°50'S 114°55'E	south of Bali	1	0.5	*Lima* sp.	naticid
480	400	8°49'S 115°00'E	south of Bali	1	1.7 x 2.3	scaphopod	naticid
489	1160	7°38'S 116°08'E	Bali Sea	1	1.7	*S. longicallis*	naticid
490	570-545	5°25'S 117°03'E	Bali Sea	1	0.7	*Limopsis* sp.	muricid
490	570-545	5°25'S 117°03'E	Bali Sea	2	0.6, 0.6	smooth-shelled bivalve	naticid
490	570-545	5°25'S 117°03'E	Bali Sea	1	0.6	spiral-lined bivalve	muricid
554	1340-1320	37°28'S 138°55'E	Australian Bight	1	0.7	scaphopod	naticid
557	680	37°13'S 138°42'E	Australian Bight	2	0.6, 1.0	mactrid bivalve	naticid
557	680	37°13'S 138°42'E	Australian Bight	1	2.2	compressed bivalve	naticid
557	680	37°13'S 138°42'E	Australian Bight	1	3.1	pectinid bivalve	naticid

Note: Where minimum and maximum exterior diameter of the borehole differed only by 0.1 mm, the maximum diameter is recorded.

Table 2. Bathymetric distribution of gastropod boreholes in valves of deep-sea *Hyalopecten frigidus* collected by the *Ingolf*.

Sta.	Depth (m)	Coordinates	Geographic region	No. boreholes	Borehole diameter (mm)	Type borehole
102	1412	66°23'N 10°26'W	northeast of Iceland	3	1.1, 1.2, 1.5	naticid
103	1090	66°23'N 8°52'W	northeast of Iceland	1	1.5	naticid
104	1802	66°23'N 7°25'W	east-northeast of Iceland	13	0.6-1.5 *	naticid
111	1619	67°14'N 8°48'W	northeast of Iceland	1	1.5	naticid
113	2465	69°31'N 8°06'W	southeast of Jan Mayen	5	0.7-1.2	naticid
117	1889	69°13'N 8°23'W	southeast of Jan Mayen	5	1.4-2.0	naticid
118	1996	68°27'N 8°20'W	southeast of Jan Mayen	15	1.0-2.0	naticid
119	1902	67°53'N 10°19'W	northeast of Iceland	5	1.5-2.0	naticid
120	1666	67°29'N 11°32'W	northeast of Iceland	5	1.0-1.5	naticid

*Range of diameter of boreholes.

Jan Mayen, at depths ranging from 1090-2465 m. Of the 144 stations surveyed during the voyage, 80 were at depths exceeding 1000 m (T. Wolff, pers. comm.). The benthic region investigated included the North Atlantic Ridge, running from eastern Greenland to Scotland and the slopes into basins north and south of the ridge (Jensen, 1912). Dr. Knudsen sent scallop valves with holes from 13 representative stations (Table 2).

The *Gosnold* shell specimens were collected off of the Middle Atlantic Bight of the eastern coast of the United States between Cape Cod, Massachusetts, and Cape Hatteras, North Carolina (Wigley and Theroux, 1981). I examined shells dredged at depths ranging from 579-1894 m from seven stations. The number of shell pieces examined per station ranged from one to about 50. Maximum depth of stations from which shells lacking holes were examined was 2725 m (unpublished *R/V Gosnold* station list, 1964) (Table 3).

Shells from each expedition were examined under low binocular microscope magnification. Maximum and minimum exterior diameter, degree of external marginal bevelling, smoothness of the wall, and cross-sectional shape (parallel to exterior surface of the shell) of each hole were recorded. Each hole was then characterized as naticid-type, muricid-type, or of other form, following the guidelines suggested by Carriker and Yochelson (1968). Identity of prey shell or fragments, when available, was taken from sample labels. Representative boreholes from the *Galathea* and *Ingolf* collections were photographed.

There was no record of which, if any, shells examined were collected alive. Also, to what extent post-mortem transport of the shells might have occurred prior to dredging, is not possible to say.

RESULTS

THE *GALATHEA* EXPEDITION

Molluscan shells with gastropod-type holes were collected from off of southern Africa to Indonesia, and off of southern Australia (Fig. 1; Table 1). They included two atypical holes in the shell of a gastropod (probably a seguenziid vetigastropod; Fig. 2) and 23 typical gastropod-type boreholes in several other species of mollusks (Figs. 3-5).

The larger of the atypical holes, although circular, possessed a rough jagged wall (Fig. 2), lacked a bevelled exterior edge and periostracum of the shell overhung the hole slightly. The overall appearance of the hole suggested penetration by means of breaking and crushing rather than by chemical dissolution and rasping. The smaller hole (Fig. 2, left) had the same appearance.

The gastropod-type boreholes (Figs. 3-5) were of two distinct types: (a) muricid-type: circular, smooth-walled, with only a slight bevel at the exterior margin (Fig. 3), and (b) naticid-type: circular, broadly bevelled exterior edge, deeply countersunk, and inner opening smaller in diameter than the outer with sharp edges (Figs. 4-5). In the

Table 3. Bathymetric distribution of gastropod boreholes in valves of deep-sea mollusks collected by the *Gosnold* off of the eastern coast of United States.

Sta.	Depth (m)	Coordinates	Geographic region	No. boreholes	Borehole diameter (mm)	Mollusk bored**	Type borehole
1255	1894	42°08'N 64°53'W	northern	1	1.1	*Nucula* sp.	naticid
1826	579	32°50'N 76°59'W	southern	3	0.6-1.1*	bivalves	naticid
				2	0.2, 0.3	bivalves	muricid
1829	1431	32°32'N 76°32'W	southern	1	0.5	*Nuculina* sp.	muricid
2202	623	41°27'N 65°56'W	northern	6	0.7-1.3*	*Astarte* sp.	inflated
2203	1795	41°35'N 65°43'W	northern	1	0.8	bivalve	? edges uneven
2204	1238	41°47'N 65°38'W	northern	1	1.0	*Nuculina* sp.	naticid
2210	802	42°02'N 65°33'W	northern	2	1.0, 1.8	*Astarte* sp.	inflated

* Range of diameter of boreholes.
** Unidentified valves and fragments of valves.

hole in Fig. 5, the radial ridges of the pectinid shell extended from the wall of the hole, suggesting that the ridges were composed of less soluble shell material than that of the remainder of the hole. In contrast, concentric ridges of the *Macoma*-like valve in Fig. 4 did not project from the wall of the hole, probably because of the generally uniform hardness of the shell material. The external surface around the hole of the pectinid valve (Fig. 5) was leeched, presenting a chalky color in a radius of about 0.5 mm, possibly the result of chemical dissolution of the surface during shell penetration.

The hole in the scaphopod shell (Table 1, Sta. 480) was oval in shape (1.7 x 2.3 mm) reflecting the roundness of the 25-mm long shell where the hole was drilled.

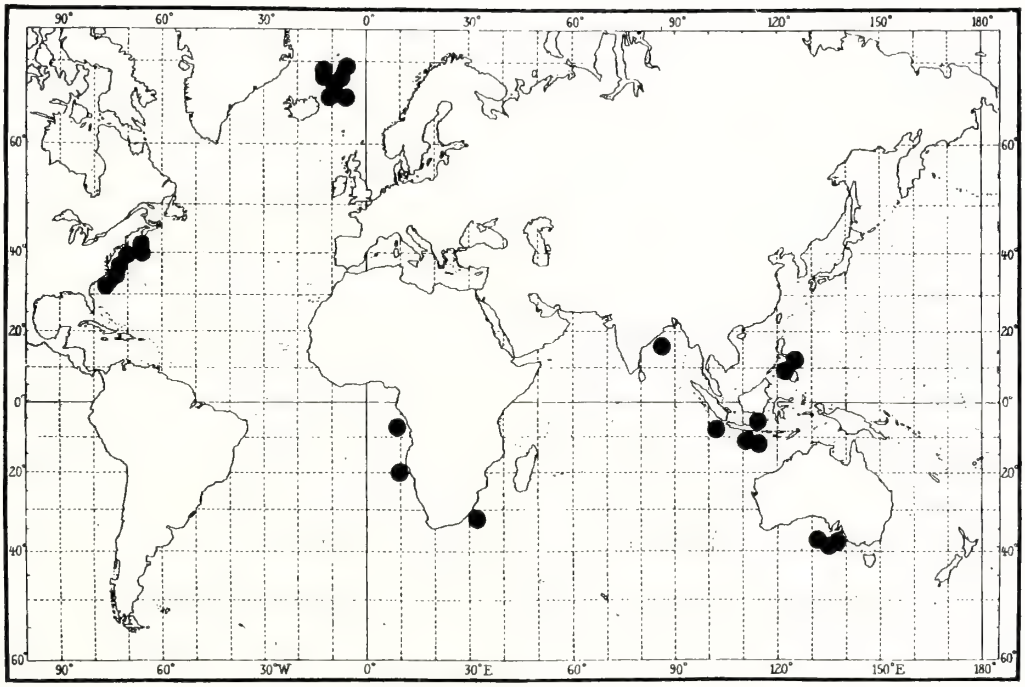
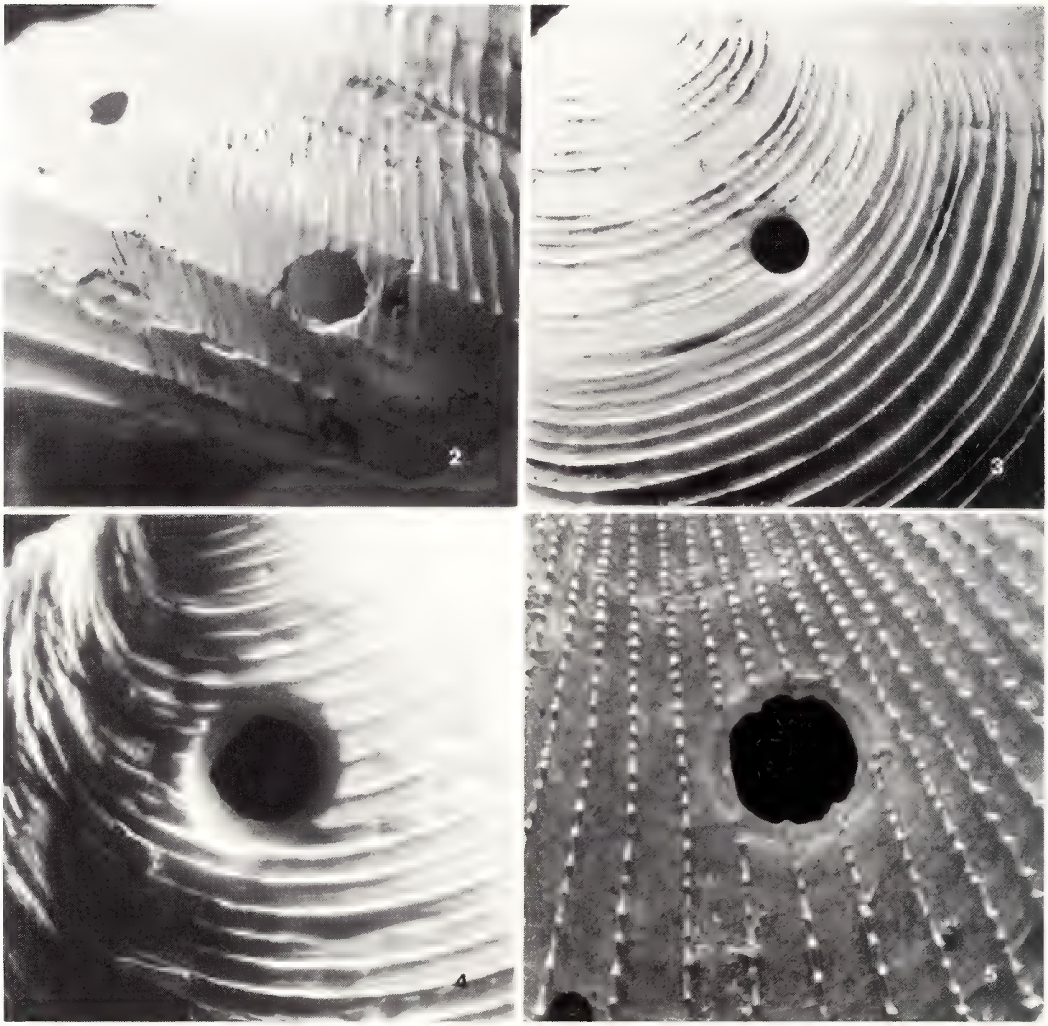


Fig. 1. Location of *Galathea* stations from off of southern Africa to off of southern Australia; *Ingolf* stations northeast of Iceland; and *Gosnold* stations off of the eastern United States, from which molluscan shells with gastropod-type boreholes were obtained.



Figs. 2-5. Gastropod bore holes. **2.** Two holes (left 0.8 mm, right 1.5 mm diameter), in shell of a presumed seguenziid vetigastropod. Below the small hole is the beginning of a second small penetration. Off of Durban, South Africa, *Galathea* Sta. 182, 3430 m. **3.** Muricid-type borehole, 1.2 mm diameter, in *Limopsis* sp., east of Cebu, Philippines, *Galathea* Sta. 436, 710 m. **4.** Naticid-type borehole, 2.9 mm diameter, in a *Macoma*-like bivalve, east of Cebu, Philippines, *Galathea* Sta. 436, 710 m. **5.** Naticid-type borehole, 3.1 mm diameter, in valve of a pectinid bivalve, Australian Bight, *Galathea* Sta. 557, 440 m.

On the whole, the range in diameter of holes was greater in naticid-type holes, 0.5-3.1 mm, than in muricid-type holes, 0.7-2.2 mm (Table 1; Fig. 6).

Most of the gastropod-type holes came from the Indonesian region (Fig. 1). Bathymetrically, most were in mollusks dredged at about 400-800 m (Fig. 6); three from an intermediate depth of about 1100-1400 m; and three, all muricid types, from 2700 m. None was found in the broad depth range of 1400-2600 m. Both naticid- and muricid-type holes were present in shallower water (400-1400 m).

THE *INGOLF* EXPEDITION

Shells of *Hyalopecten frigidus* from nine stations located northeast of Iceland (Fig. 1; Table 2), included 53 typical naticid-type boreholes (Fig. 7). There were no muricid-type boreholes.

The valves of these pectinids were thin; holes were circular, smooth, broadly bevelled on the external margin, and narrowed to a small inner opening with slightly rough edges. Small radial ridges of the shell were reflected in the wall of the holes (Fig. 7). No leeching of the shell surface was evident around the external surface of the holes. Although the degree of bevelling varied widely from one hole to another, pronounced bevelling was characteristic of all holes, even though the pectinid valves were extremely thin.

The range in external diameter of holes was 0.6-2.0 mm (Table 2; Fig. 8). The largest holes were in valves dredged at depths between 1900 and 2000 m.

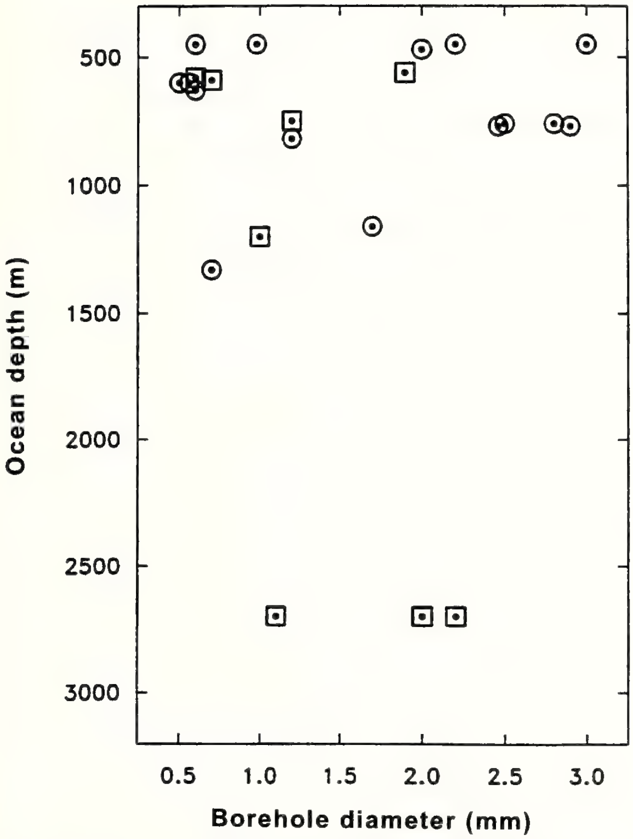


Fig. 6. Bathymetric distribution of naticid-type and muricid-type boreholes in *Galathea* mollusks. (circles, naticids; squares, muricids).

Bathymetrically, most valves with holes were distributed approximately between 1400 and 2000 m; only two came from about 2500 m. Maximum numbers were dredged at depths ranging from 1700-2000 m (Fig. 8).

THE *GOSNOLD* SURVEY

Molluscan shells with gastropod-type holes were taken from dredgings at 13 stations off of the eastern United States from Massachusetts to South Carolina (Fig. 1; Table 3), and included a total of 17 holes. Of these, three were of the muricid-type, five of the naticid-type, eight were inflated (or barrel-shaped), and one was atypical.

Muricid-type holes were distinctly cylindrical with only a slight external bevel (similar to Fig. 3). Naticid-type holes were distinctly shallowly parabolic (because of the thinness of the valves), with a broadly bevelled exterior edge and inner opening smaller in diameter than the outer, with irregular sharp edges (similar to Fig. 4). In the case of the hole in a *Nucula* sp. valve (Sta. 1255) which was recently killed, the periostracum of the valve hung slightly onto the sides of the broad bevelling of the hole.

Inflated holes were present only in valves of *Astarte* sp. The diameter of each hole was enlarged centrally and decreased toward the exterior and interior openings, giving the hole the shape in section of a small barrel (similar to Carriker and Yochelson, 1968: figs. 12-13). The external margin was only slightly bevelled.

The range in external diameter of naticid-type holes (0.6-1.1 mm) was less than that of the inflated holes (0.7-1.8 mm), whereas that of muricid-type holes was only 0.2-0.5 mm (Table 3).

Bathymetrically, muricid-type holes were found in shells dredged from 579-1431 m, and naticid-type holes from 579-1900 m. Inflated boreholes were dredged at depths of 623-802 m, and were found in the middle to northern geographic region off of the coast (Table 3; Fig. 9).

DISCUSSION

Muricid-, naticid-, and inflated-type holes in valves of mollusks dredged during the *Galathea, Ingolf*, and *Gosnold* voyages from depths as great as 2690 m are characterized by features so strikingly similar to those of boreholes drilled by shallow-water gastropods that they can properly be classified as gastropod boreholes. Until recently, the distribution of identified gastropod boreholes was known principally from intertidal and shallow subtidal benthic regions; the present study demonstrates that they occur bathymetrically into at least bathyal depths.

How much deeper into abyssal and hadal regions

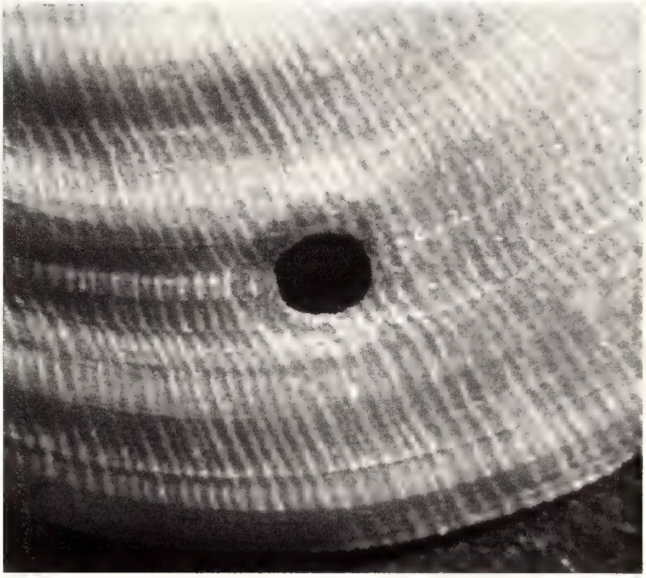


Fig. 7. Naticid-type borehole, 2.0 mm diameter, in *Hyalopecten frigidus*, southeast of Jan Mayer, *Ingolf* Sta. 117, 1889 m.

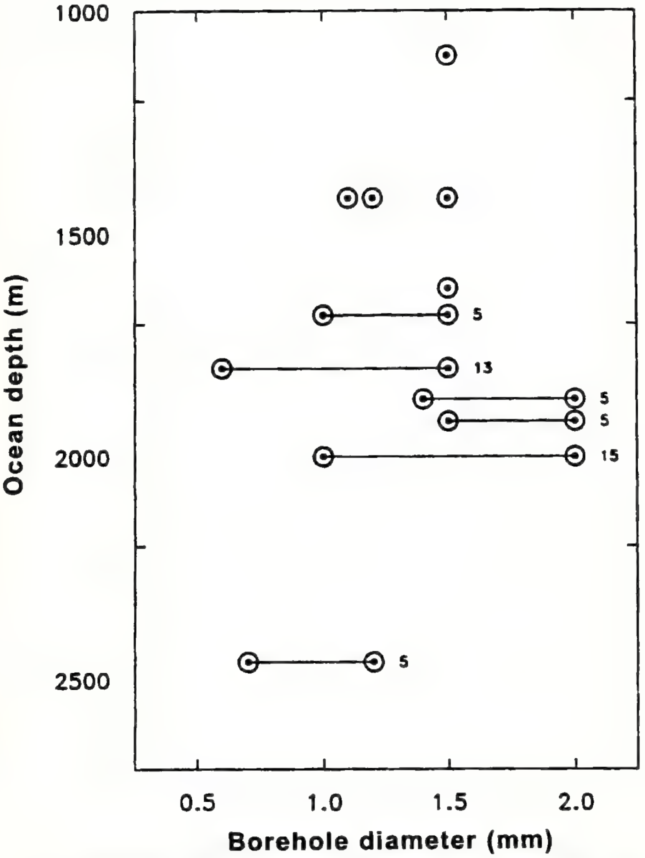


Fig. 8. Bathymetric distribution of naticid-type boreholes in *Hyalopecten frigidus* from the *Ingolf*.

boring gastropods occur, remains for future explorers to determine. The bathymetric distribution of mollusks in general, however, provides suggestions (Zezina, 1997). Deep-sea gastropods and bivalves, characteristically small (roughly 0.7-28 mm long), thin-shelled, and with reduced ornamentation (Clarke, 1962b), for example, have been dredged at all the bathyal, abyssal, and hadal depths explored to date. According to Bruun (1957a), apogastropods were taken in the Kurile Trench at 8330-8050 m; Wolff (1960) reported that gastropods and bivalves were dominant groups in 13 deep-sea trenches investigated at hadal depths exceeding 6000 m. In his exhaustive tabulation Clarke (1962a, b) listed all species of mollusks validly recorded from depths of 1830 m or more; and Knudsen (1970, 1979) recorded species of deep-sea bivalves from both abyssal and hadal regions.

The bathymetric distribution of species of Naticidae and Muricidae reported from the deep sea provides further suggestions. Schepman (1909) listed 37 species of *Natica* from bottoms in the shallower bathyal region close to *Galathea* stations from whence bored shells were collected for the present study (J. Knudsen, pers. comm.). Wolff (1960) mentioned one to three species of Naticidae - but no Muricidae - taken at 6860 m in the Kurile-Kamchatka Trench (see also Bruun 1957a). Egorov (1993) listed *Abyssotrophon hadalis* (Sysoev, 1992) from the same trench at a depth of 6475-7230 m, as well as other species of the same genus at slightly shallower depths in this same trench. Clarke (1962a, b) recorded 21 species of naticids and 14 species of muricids from bathyal regions. And Theroux (1983), in a list of gastropods off of the eastern coast of the United States, included ten boring species. Two of the naticid species, *Natica clausa* Broderip and Sowerby, 1829, and *Euspira pallida* (Broderip and Sowerby, 1829) were dredged in water as deep as 1715 and 1170 m, respectively. McLean (1997) reported 18 caenogastropod families from the northeastern Pacific with depth records of 800 m and deeper. Because the majority, possibly all, of the species of shallow-water muricids, naticids, and trophonids studied to date are borers (Carriker, 1981; Kabat, 1990; Egorov, 1993), it is highly likely that species of these families occurring in the deep sea are likewise shell penetrators. Other deep-sea gastropod families containing boring species, such as capulids and ranellids, probably also exist.

It is puzzling that Knudsen (1964) named no species of Muricidae or Naticidae in a list of several gastropod

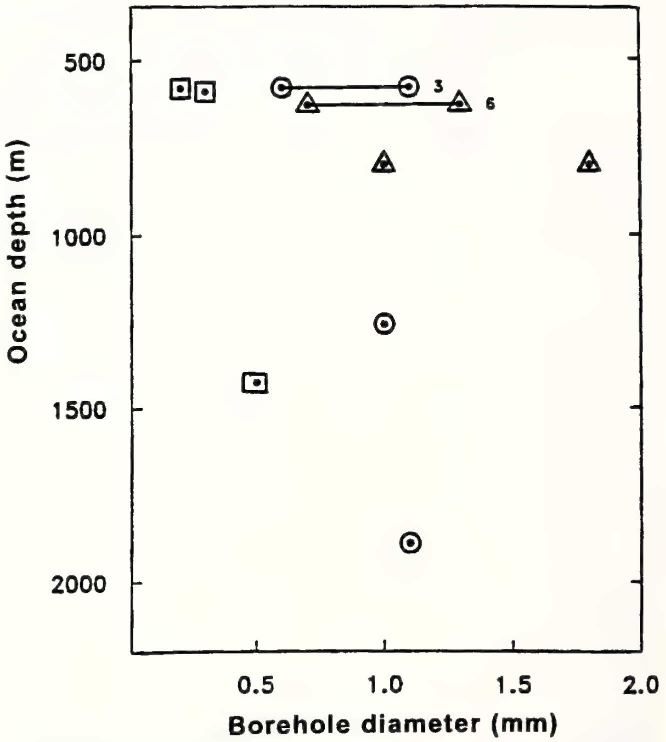


Fig. 9. Bathymetric distribution of gastropod-type boreholes in *Gosnold* mollusks. (circles, naticids; squares, muricids; triangles, inflated boreholes).

families from hadal depths exceeding 6000 m. The reported absence of these families could be explained by a rapid decrease in faunal diversity beyond the abyssal zone (Knudsen, 1956), by spotty distribution of gastropods on the bottom, by limited dredge size, and by the non-cosmopolitan often endemic distribution of molluscan species in deep-sea basins (Clarke, 1962a, b).

The general paucity of muricid species reported to date in the deep sea is reflected in the low number of muricid boreholes (11) compared to the relatively high number of naticid holes (73; Table 4) in molluscan shells from the three voyages. The low density of reported muricid species (Wolff, 1960; Clarke, 1962a; Theroux, 1983) is probably a result of the relative scarcity of hard surfaces in the deep sea or limited dredging on hard surfaces (incompatible with dredges and trawls), rather than the effect of other environmental factors associated with depth. In shallow water, at least, soft substrata tend to inhibit muricid reproduction (Carriker, 1981). That some muricid-type boreholes do occur could be explained by the fact that trophonid gastropods, for example, are found on substrata ranging from loam, silt, and sand, to pebbles and rocks (Egorov, 1993).

The occurrence of naticid boreholes in pectinid valves collected during the *Ingolf* expedition is somewhat inconsistent with the usual habit of shallow-water naticids of drilling prey while buried under the sediment-seawater interface. Although naticids can attack bivalves on the surface of the sea floor (Carriker, 1981; Kabat, 1990), this is probably an uncommon behavior. Also, because naticids generally enfold prey within their foot, flattened bivalves like pectinids could hinder the shell-penetrating process. A possible explanation is that these borers were species of *Boreotrophon* that bore naticid-type holes. They possess a small foot and are able to cling to the flattened valves of pectinids; and they occur in the Iceland area (G. Vermeij, pers. comm.).

Shallow-water muricids, on the other hand, with their relatively small foot, are definitely not sedimentary burrowers and live on the surface of firm substrata where they can attack epifaunal bivalves and barnacles. If deep-sea muricids likewise possess a small foot, one would assume that they, too, dwell on firm surfaces on, or extending above, the sediment. Thus on dredgeable open sea floors where there is a scarcity of hard surfaces, few muricids would be expected, although some species of trophonids could be present there (Egorov, 1993).

Table 4. Comparison of gastropod borehole data from the three expeditions.

	Galthea	*Ingolf*	*Gosnold*
No. muricid-type boreholes	8	0	3
No. naticid-like boreholes	15	53	5
No. barrel-shaped boreholes (inflated)	0	0	8
Range borehole diameter (mm)	0.5-3.1	0.6-2.0	0.2-1.8
Range station depth at which boreholes found (m)	440-2690	1090-2465	579-1894

The shape of inflated boreholes in the valves of *Astarte* sp. (Table 3) is something of a paradox. This type of penetration was found only in valves of this species and only in collections dredged by the *Gosnold*. The external diameter of the holes ranged from 0.7-1.8 mm (greater than that of deep-sea naticids, 0.6-1.1 mm). The larger diameter of inflated boreholes than that of deep-sea muricid boreholes (0.2-0.5 mm) could suggest that the former were drilled by a snail species other than a muricid. The borehole drilled in *Chione cancellata* (Linné, 1767) by *Chicoreus florifer* (Reeve, 1846), in the laboratory was also large (range of external diameter 1.9-2.3 mm) (see Carriker and Yochelson 1968: pl. 2, figs. 12-13).

An explanation for the inflated middle diameter of the interior of the borehole in *Astarte* sp. and *Chione cancellata* is unknown. Possibly the quality of shell material between exterior and interior surfaces of the valve is softer or becomes altered with time, allowing more rapid dissolution of the shell there during the chemical-mechanical boring process (Carriker, 1981). That composition of shell does make a difference in the diameter of a borehole, was demonstrated in boreholes drilled by *Eupleura caudata* (Say, 1822) from the New Jersey coast in valves of *Crassostrea virginica* (Gmelin, 1791) (see Carriker and Yochelson 1968: pl. 1, figs. 7-8). The diameter of these boreholes was inflated within chalky shell layers in marked contrast to that through harder foliated shell layers (Carriker, 1996). Sectioning of *Astarte* sp. boreholes and testing the hardness of the shell strata have not been done, but could provide an explanation for this form of hole.

The atypical jagged holes (Fig. 2) in the valve of the vetigastropod (Sta. 192, 3530 m; Table 1) are also a puzzle. No known shallow-water boring gastropod excavates this kind of hole in the shell of molluscan prey.

The exterior diameter of boreholes excavated by different adult species of boring gastropods varies widely. In our collection (Carriker and Yochelson, 1968), exterior diameters range from 0.6-10 mm. Boreholes drilled by snails newly emerged from the egg capsule, are 0.1 mm or less in diameter (Carriker, 1957). Boreholes in the present specimens (Table 4) ranged from 0.2-3.1 mm in outer diameter, dimensions conspicuously less than those drilled by shallow-water boring gastropods. This is not surprising, as maximum sizes of shallow-water (sublittoral) naticids and muricids are substantially larger than those of any bathyal or abyssal species. The size of the borehole does not necessarily indicate the age of the driller, although as a general rule smaller holes are bored by smaller snails, either small juveniles or small adults.

The present report does leave many questions unanswered. These include the maximum bathymetric distribution of gastropods that drill shelled prey, the distribution of snail borers into deep-sea polar regions (Aitken and Risk, 1988), the apparent relative scarcity of muricid boreholes, the identity and characteristics of snail species that bore holes and their method of penetrating shell. Whether boring snails dwell in the deepest reaches of hadal regions still remains unclear, although incidental observations reported earlier indicate that they could. The fact that prey shells dissolve fairly rapidly after death of the mollusk below calcium carbonate compensation depths, decreases the likelihood of evidence of drilling predation in the deep sea; gastropods with accessory boring organs at abyssal and hadal depths would be more accurate predictors of drilling predation than the absence of bored shells. The definitive identification of deep-sea boring snails, however, will require observation of the living animals in the act of drilling prey.

The observation that deep-sea boreholes examined in the present study are similar to those drilled by shallow-water boring gastropods supports Clarke's (1962a, b) hypothesis that mollusks invaded the deep sea from shallow water during the recent geologic past.

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Redescription of *Atys macandrewii* E. A. Smith, 1872, an amphiatlantic cephalaspidean

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Abstract: *Atys macandrewii* E. A. Smith, 1872 (Gastropoda: Cephalaspidea: Haminoeidae), originally described from the Canary Islands, is redescribed from specimens collected in Cape Verde and in the Canary Islands, in comparison with the type material. Living coloration is described, and the radula, jaws, and gizzard plates are studied by scanning electron microscopy. Synonymy between Smith's species and the western Atlantic *A. lineata* Usticke, 1959, as previously suggested by Marcus (1970), is confirmed here through study of their respective type material.

Key words: Cephalaspidea, Haminoeidae, *Atys macandrewii*, redescription, Atlantic waters

The genus *Atys* Montfort, 1810, is characterized, according to Pilsbry (1895), by an involute, globose-oval to subcylindrical-oval shell, with the spire concealed and a short columella. The aperture is as long as the shell and produced above the vertex, with the lip rising from the center of the vertex.

Atys macandrewii (originally *M'andrewii*) was described by Smith (1872) from the Canary Islands on the basis of empty shells collected by R. M'Andrew at Lanzarote. Smith's original description was brief and referred only to the shell, "at once recognized by the numerous lacteous bands upon a pellucid ground."

Since its original description, *Atys macandrewii* has been mentioned in the literature only in a few works. Odhner (1932), Nordsieck (1972), and Nordsieck and García-Talavera (1979) repeated the record from Canarian waters, and the species is also known from Madeira (Nordsieck, 1972; Burn, 1978), Selvagens (Talavera, 1978), and the Azores (Nordsieck and García-Talavera, 1979; Mikkelsen, 1995); thus present material constitutes the first record from the Cape Verdes.

Marcus (1970) considered this species as the senior synonym of the western Atlantic *Atys lineata* Usticke, 1959 (originally described from the Virgin Islands), and recorded it from Brazil, pointing out its amphiatlantic distribution and providing a quite complete description of the shell, gizzard plates, and penis. Here, examination of specimens collected in the Cape Verde archipelago and in the Canary Islands, and comparison with the type material (in The Natural History Museum, London), allow redescription of the species. An external description of living specimens is given for the first time, and the radula, jaws, and gizzard plates are studied by scanning electron microscopy.

MATERIAL AND METHODS

The specimens studied in this paper were collected during scientific expeditions or provided by the following institutions: AMNH, American Museum of Natural History, New York; MNCN, Museo Nacional de Ciencias Naturales, Madrid; NHM, The Natural History Museum, London.

Description of living Cape Verde specimens is interpreted from original notes and drawings made by the collectors. Preserved specimens were dissected and hard parts of the digestive system were studied by scanning electron microscopy.

SYSTEMATIC SECTION

Genus *Atys* Montfort, 1810
Type species *Bulla naucum* Linné, 1758

Atys macandrewii E. A. Smith, 1872

Atys M'Andrewii E. A. Smith, 1872: 346.
Atys lineata Usticke, 1959: 85, pl. IV, fig. 15.
Atys (*Alicula*) *macandrewi* Smith. Odhner, 1932: 24, pl. I, fig. 20.

Material examined

Atys macandrewii syntypes: 9 empty shells from the Canary Islands (Lanzarote), 1.79-4.46 mm length, NHM 1855.4.4.282.

Material from Sal Island, Cape Verde: Palmeira, 11 specimens, 3.65-5.27 mm length; Fontona, 7 specimens, 3.67-4.96 mm length. All were dried specimens that were rehydrated using 5% trisodium phosphate. All were collected by dredging in shallow waters during the First Iberian Expedition to Cape Verde, August 1985; voucher specimens are available as MNCN 15.05/21436 and 15.05/21437.

Material from Tenerife, Canary Islands: 4 specimens, 5.53-6.70 mm length, collected by dredge in shallow waters, February 1996.

Atys lineata: one paratype (empty shell), 5.46 mm length, from Ham Bay, St. Croix, Virgin Islands, AMNH 270411 (Louis and Lorraine Schwartz, donors); 17 empty shells, 2.72-5.58 mm length, from the type locality, AMNH 193881 (Gordon Nowell-Usticke coll.); 3 empty shells, from Charloteville Bay, New Providence, Bahamas, 4.15, 5.17, and 5.22 mm, AMNH 277638.

Original description

Smith's original description of *Atys macandrewii* was translated by Pilsbry (1895: 273) as follows: "shell elongate-ovate, truncated above, pellucid, encircled by numerous narrow milky bands, one in the middle wider; transversely distantly striated at top and base, the interstice smooth; vertex excavated, bounded by an acute margin. Aperture narrow, produced a little above the vertex, a little dilated and effuse at the base; lip thin, inserted in the middle of the vertex and sinuated there; columella short, thickened, hardly twisted; umbilical region slightly perforated." According to Pilsbry, this species was easy to distinguish by "the numerous lacteous bands upon a pellucid ground" on the shell.

Redescription

The type material of *Atys macandrewii* consists of nine empty shells, two of them still glued to a piece of black paper (Figs. 1A-C). Although there is no locality designated on the label of this material, it is Lanzarote (Lancerote) according to the original description. All of the type specimens have transverse white bands, and some very clearly show this species-specific character, *e. g.* the smallest one (Fig. 1C) and also a larger specimen (Fig. 1A).

In all of the specimens we have examined, the involute shell is oval and elongate, with a narrow aperture, transparent, and provided with opaque white spiral bands (Figs. 1, 2). The opaque white bands are always present, but their density varies among specimens. Usually there is a broad band in the middle and narrow ones toward the ends, although some specimens show several fine white bands in the central area instead of a single broad one (Fig. 1E); another specimen has opaque white bands on the shell, but not disposed in the middle (Fig. 1F).

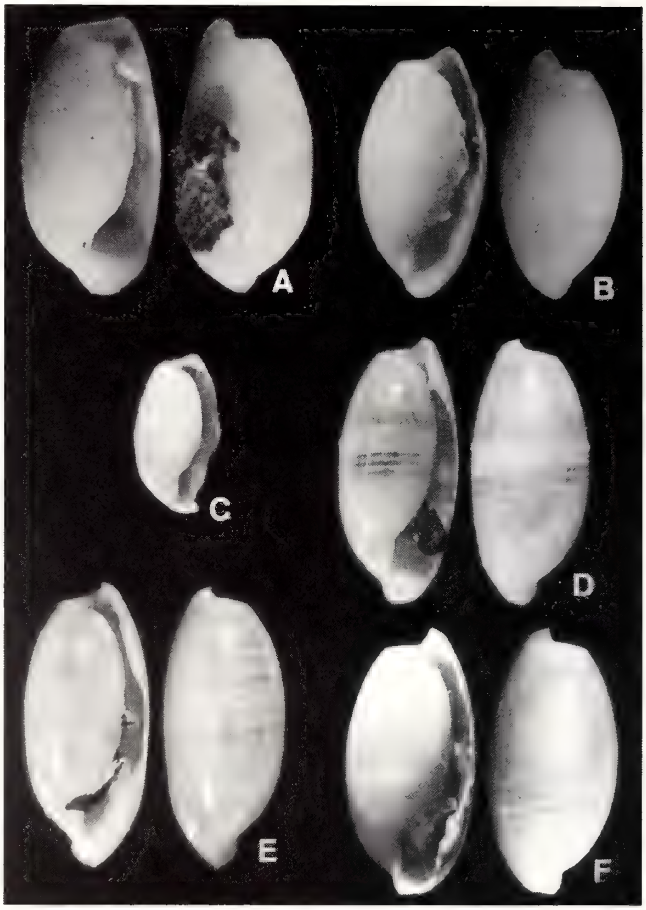

Fig. 1. *Atys macandrewii*. **A**. Syntype, NHM 1855.4.4.282 (3.44 x 1.58 mm). **B**. Syntype, NHM 1855.4.4.282 (4.46 x 2.16 mm). **C**. Syntype, NHM 1855.4.4.282 (1.79 x 0.99 mm). **D**. Shell from Fontona, Cape Verde (4.72 x 2.12 mm). **E**. Shell from Palmeira, Cape Verde (4.61 x 2.04 mm). **F**. Shell from Tenerife, Canary Islands (5.53 x 2.69 mm).

The shells are also sculptured with spiral grooves on their anterior and posterior thirds (usually 7-10 grooves in the posterior part and 10-12 anteriorly), whereas the central area is smooth (Fig. 3B). The range of variation of the shell length and width is recorded in Table 1.

In living specimens of *Atys macandrewii*, the ground color of the body is pellucid white with irregular dots and spots of white opaque pigment. The cephalic shield is large, bilobed at its posterior end, and disposed over the anterior part of the shell (Fig. 3A). The parapodial lobes are short, not meeting mid-dorsally.

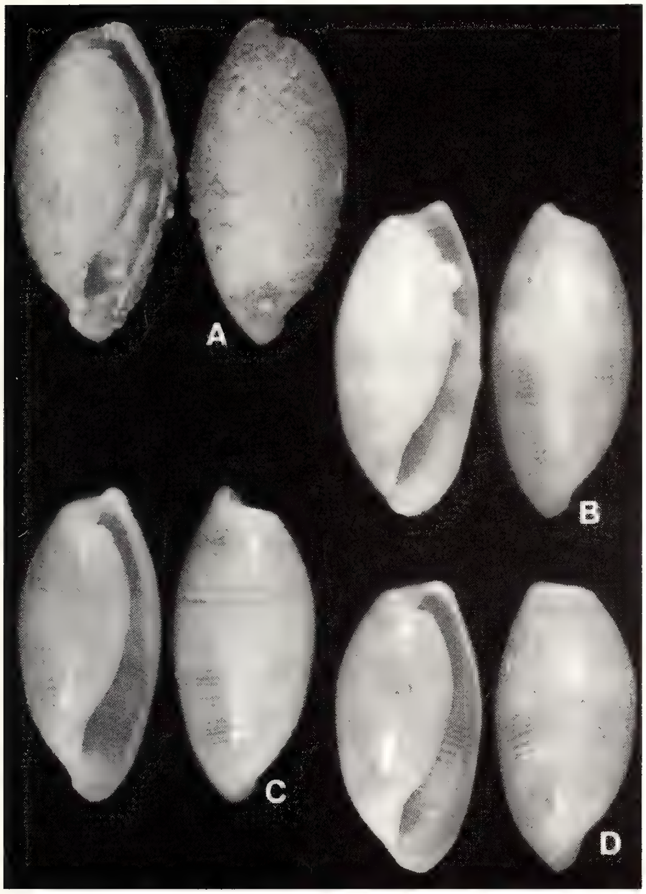

Fig. 2. *Atys lineata* (= *A. macandrewii*). **A**. Paratype, AMNH 270411 (5.46 x 2.71 mm). **B**. Shell from the type locality, AMNH 193881 (5.58 x 2.49 mm). **C**. Shell from the type locality, AMNH 193881 (3.63 x 1.62 mm). **D**. Shell from the Bahamas, AMNH 277638 (5.22 x 2.52 mm).

In four examined radulae, the formula was 7.1.7 (Fig. 4A), with 19-21 rows. The rachidian tooth is wide and low, with a cusp centrally notched and divided in two lobes, each bearing 12-15 sharply pointed denticles (Fig. 4B). The first lateral teeth are broad at their bases, with a single long cusp bearing a few external denticles (Figs. 4C-D). Toward the margins the teeth become shorter and smaller, and they are provided with more numerous external denticles (Figs. 4E-F).

Jaw rodlets (Figs. 4G-H) are short (less than 10 µm), wide, and flattened toward their free edge, bearing 17-20 finger-shaped denticles.

The three oval gizzard plates are equal in size, each with about 20 transverse folds, each of these showing a single row of spiny cones (Figs. 5A-D). Marcus (1970: 936) described the gizzard plates of one Brazilian specimen as measuring 590 by 290 µm, and "provided with 25 crests with a single row of spines."

Comments on *Atys lineata*

The examined material of *Atys lineata* consists of empty shells (Fig. 2), with general morphology identical to that described for *A. macandrewii*. The specimen designated by Usticke as paratype and examined here (Fig. 2A) is a badly eroded shell, in which the opaque white spiral bands are not visible.

The original description of *Atys lineata* given by Usticke (1959: 85) is brief and only refers to the shell, as follows: "The white background bears many colorless spiral lines, which appear quite dark on a white background... These spirals seem to be grouped together, usually having a plain space in the middle." It must be pointed out that Usticke saw clear lines on a white shell, while Smith (1872) described white bands on a clear shell.

Usticke's original description also stated that the shell of *Atys lineata* lacks the incised spiral lines found in other *Atys* species, such as *A. sharpi* Vanatta, 1901; this was repeated by Warmke and Abbott (1961: 143). Nevertheless, in the shells from the type locality examined here (Figs. 2B-C), the spiral lines are well-developed and clearly visible.

DISCUSSION

The original description of *Atys macandrewii* referred only to shell morphology, and soft parts of the

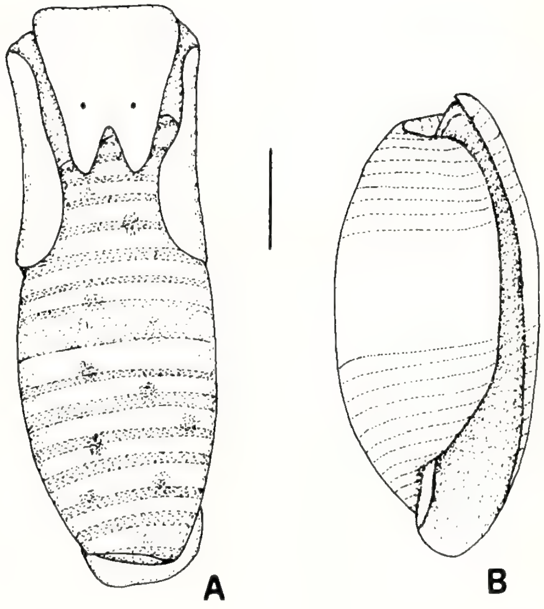

Fig. 3. *Atys macandrewii*. **A**. Dorsal view of a living specimen. **B**. Drawing of the shell showing the location of spiral grooves. Scale bar = 1 mm.

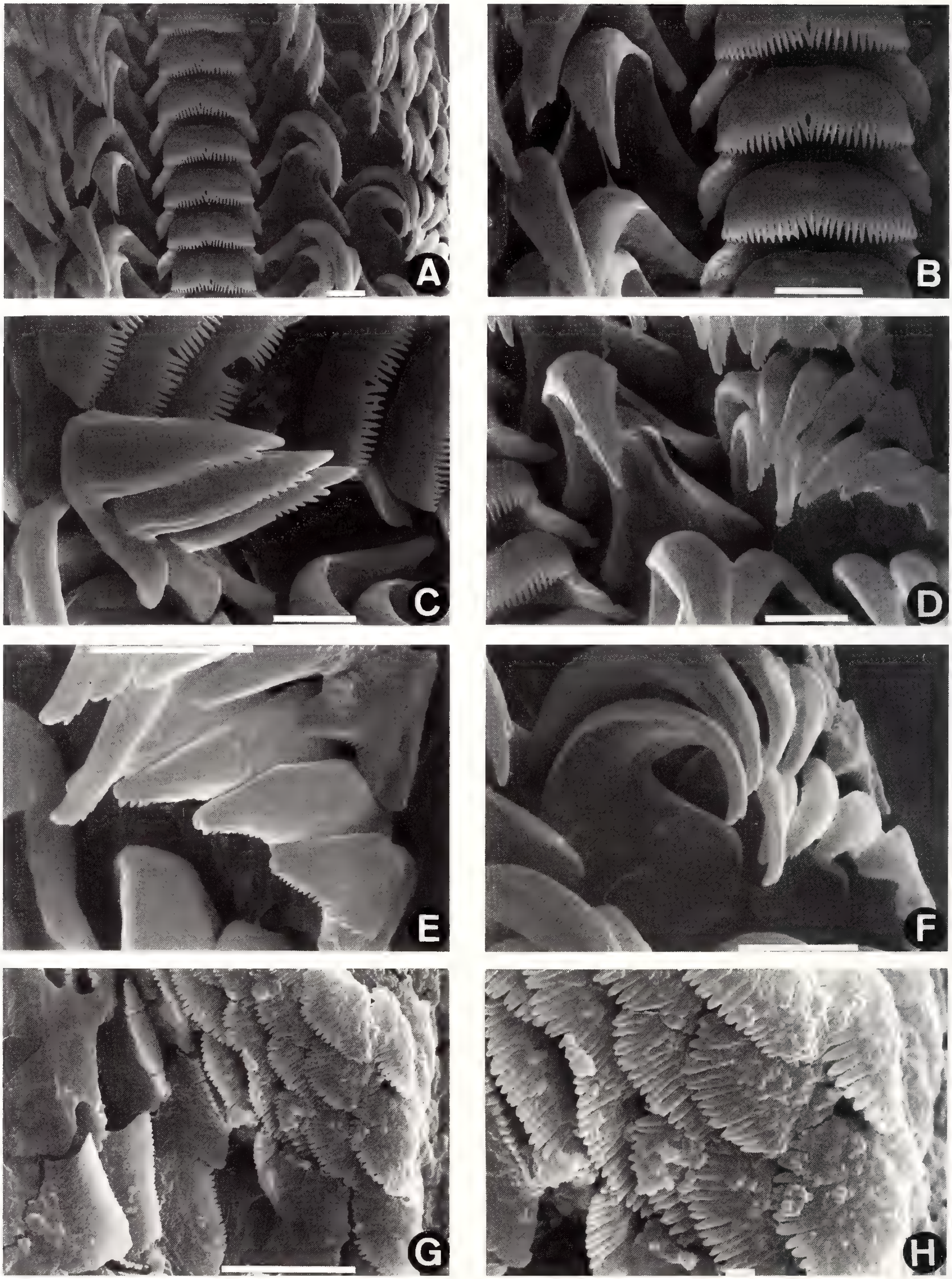

Fig. 4. *Atys macandrewii*, Palmeira, Cape Verde, radulae and jaws. **A**. General view of the radula. **B**. Rachidian tooth. **C**. First lateral tooth. **D**. Lateral teeth. **E**. Lateral teeth toward the margin. **F**. Outer lateral teeth. **G**. Jaw elements. **H**. Detail of jaw elements. Scale bars = 10 μm (A-G), 1 μm (H).

Table 1. Range of variation of shell length (L) and width (W) (in mm) in *Atys macandrewii* from several localities (* material labelled as *A. lineata*).

Locality	N	Shell Dimensions	W/L Ratio	Source
Brazil	—	5.20 x 2.80 to 7.30 x 3.60	0.49 to 0.54	Marcus, 1970
Virgin Islands *	18	2.72 x 1.28 to 5.58 x 2.49	0.45 to 0.47	AMNH 270411 and 193881
Bahamas *	3	4.15 x 1.90 to 5.22 x 2.52	0.46 to 0.48	AMNH 277638
Canary Islands (type material)	9	1.79 x 0.99 to 4.46 x 2.16	0.49 to 0.55	NHM 1855.4.4.282
Canary Islands (Tenerife)	4	5.53 x 2.69 to 6.70 x 3.24	0.48 to 0.49	present study
Cape Verde	18	3.65 x 1.67 to 5.27 x 2.37	0.45 to 0.46	present study

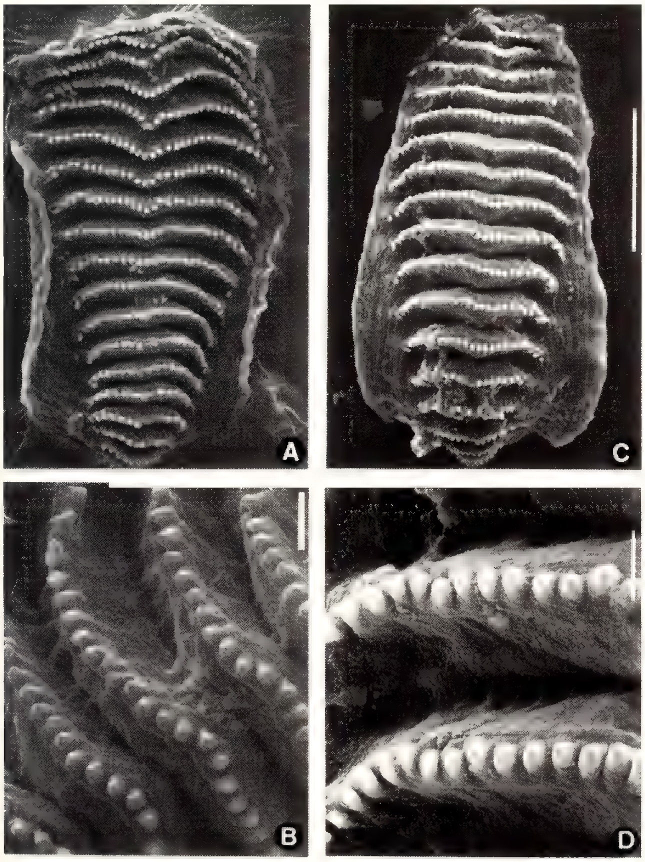

Fig. 5. *Atys macandrewii*, gizzard plates. **A**. From specimen from Palmeira, Cape Verde, general view. **B**. Same as A, detail. **C**. From specimen from Tenerife, Canary Islands. **D**. Same as C, detail. Scale bars = 10 μm (A, B, D), 100 μm (C).

body were not taken into account. We attribute our specimens from Cape Verde and from Tenerife to Smith's species on the basis of their shells, provided with several clearly visible transverse white bands, a character not present in any other species of the genus.

In a Brazilian specimen, Marcus (1970: 936) described the partly evaginated male organ as "... a muscular papilla and entally a bipartite duct, opening into two vesicles, the shorter of which has a glandular epithelium and corresponds to the caecum; the longer stores sperm." Unfortunately, we were unable to verify the penial morphology in dried specimens from the Cape Verde Islands.

As previously mentioned, Marcus (1970) recorded *Atys macandrewii* from Brazil, considering this species as a senior synonym of *A. lineata*, with type locality in the Virgin Islands, and also recorded from the Caribbean Sea and Curaçao. According to Marcus, the transverse white bands of the shell make *A. macandrewii* "an unquestionable species." In fact, shell morphology of the specimens of *A. lineata* examined here is identical to that of our eastern Atlantic specimens.

Comparison of the type material of *Atys macandrewii* and *A. lineata*, and other shells from both Atlantic shores allows us to confirm their synonymy and reaffirm the amphiatlantic character of this species. The presence of wide and well-defined white transverse bands in the shell is a character not present in other Atlantic and Mediterranean species of this genus, such as *A. diaphana* (Aradas, 1840), *A. caribaea* (Orbigny, 1841), *A. blainvilleana* (Récluz, 1843), *A. brocchii* (Michelotti, 1847), *A. jeffreysi* (Weinkauff, 1866), *A. guildingi* Sowerby, 1869, *A. canariensis* Smith, 1872, *A. riiseana* Mörch, 1875, *A. sandersoni* Dall, 1881, *A. sharpi*, *A. cylindricella* Usticke, 1971, and *A. globulinus* Nordsieck, 1972.

Although Abbott (1974: 320) considered *Atys lineata* as a synonym of *A. caribaea*, the latter is clearly a different species, its shell uniformly white in color and showing close spiral lines at both ends. All species included in the genus *Atys* have been described mainly on the basis of shell morphology, whereas soft parts have been poorly studied. The systematic status of many of these species requires careful review, as does the amphiatlantic character of some of them.

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Induction of polyploidy and embryonic development of the abalone, *Haliotis diversicolor*, with temperature treatment

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Abstract: The effect of blocking the release of the first polar body (PB1) or second polar body (PB2) with 3°C cold shock treatment on the development and ploidy of embryos was studied in the small abalone, *Haliotis diversicolor* (Lischke 1846); Groups of PB1 eggs were treated for 5, 10, 20, or 40 min, and resulted in 21 ± 3.0%, 17 ± 3.5%, 7 ± 2.0%, and 0% normal gastrulae, respectively. The same treatment of PB2 eggs produced 12 ± 2.6%, 15 ± 2.6%, 3 ± 2.5%, and 0% normal gastrulae, respectively. The percentage of normal embryos in the control group was 68 ± 2.3%. Significant differences in percent normal embryos (by ANOVA and Duncan's analysis) were found between the treatments and the control ($F_{3,16} = 19.28$, $p < 0.001$) but not between equivalent treatments in the PB1 and PB2 groups. In the control groups, 41.8% of the embryos were diploid, while spontaneous haploids (9.0%), triploids (7.5%), and aneuploids (41.7%) also occurred. When the percentages of diploids, triploids, and aneuploids in the treated groups were compared with the control, there was a significant difference with respect to the diploid group ($F_{4,4} = 6.88$, $p < 0.05$) but not the triploid ($F_{4,4} = 1.60$, $p > 0.05$) or aneuploid ($F_{4,4} = 3.05$, $p > 0.05$) groups. Comparison (using Duncan's analysis) of the treatments and the control of the factors of time and polar body in relation to diploidy showed a significant difference only in the 10 min group of PB1 and PB2 ($p < 0.05$) but no variation between PB1 and PB2 ($p > 0.05$). The results show a positive relationship between the length of immersion time at 3°C and the number of abnormal embryos, and no significant relationship between 10 min immersion and the development of tripoidy. This study illustrates that the formation of various levels of ploidy during chromosome segregation is random. No differences in forming various ploidy levels were detected between the PB1 and PB2 groups.

Key words: polar bodies, ploidy, development, aquaculture, Haliotidae

Recently, much discussion in the aquacultural sciences has focused on the potential of chromosome manipulation techniques, most specifically the artificial induction of triploidy (three sets of chromosomes in each cell). Two features of triploids are advantageous: (1) The nuclei of triploid cells are larger to accommodate increased DNA in the nucleus, and this leads to a concomitant increase in overall cell size. (2) Triploids have poorly developed gonads and produce far fewer gametes than diploids, resulting in overall larger individuals. This is in contrast to diploid organisms which grow until sexual maturity is reached when growth slows down or stops as a large proportion of energy becomes devoted to the production of eggs or sperm.

Previous investigations have demonstrated the application of chemical agents, temperature (heat or cold), or hydrostatic pressure to induce triploidy (Stanley *et al.*, 1981, 1984; Chaiton and Allen, 1985; Arai *et al.*, 1986; Quillet and Panelay, 1986; Dowing and Allen, 1987; Yamamoto *et al.*, 1988; Allen *et al.*, 1989; Stephens, 1989; Kudo *et al.*, 1991). Triploids have been induced in a variety of fish and mollusks (Longo, 1972; Arai *et al.*, 1986; Beaumont and Fairbrother, 1991).

The small abalone, *Haliotis diversicolor* (Lischke, 1846), is currently raised in commercial hatcheries in Taiwan (Chen and Yang, 1979). Triploid abalone could be an important part of the abalone culture industry if they can be shown to have a faster growth rate than diploid individuals. Abalone growers wish to produce the maximum amount of flesh so preventing the onset of sexual maturity using triploidy is desirable. To be commercially beneficial, the induction technique must result in a high percentage of triploids and a high percent survival. Possible factors affecting these two parameters include the induction method, the polar body that is blocked, the animal species, conditions during induction, start time and duration, ambient temperature, and the quality of the eggs and sperm.

In the Pacific abalone, *Haliotis discus hannai* (Ino, 1980), Arai *et al.* (1986) reported that 3°C cold shock produced high percentages of triploids. Kudo *et al.* (1991) used the same inducing conditions with *H. diversicolor diversicolor*, but did not produce the same results. The latter authors provided a more detailed analysis of early embryos which could partly account for this discrepancy. Stanley *et al.* (1984) found that meiosis I triploid oysters (inhibiting the first polar body; PB1) grew better than

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meiosis II triploids (inhibiting the second polar body; PB2), however, their observation continues to be controversial (Beaumont and Fairbrother, 1991) and the induction of PB1 and PB2 triploids continues to be of interest. In this study, karyological analyses were used to determine ploidy of gastrula-stage embryos of *H. diversicolor* following blocking of PB1 and PB2 with different exposure times to 3°C cold shock treatment. These findings should help clarify the optimum conditions for the manipulation of triploidization in this species.

METHODOLOGY

Gamete preparation

Parental abalone were cultured from the seed of artificial propagation and broodstock in a cement pond (1.5 x 4 x 1.0 m) at the Tainan Station of the Taiwan Fishery Research Institute (Yang and Ting, 1987, 1988). 20 male and 20 female abalone (shell length 6-7 cm; 25-30 g) were placed separately into 24 l plastic containers (40 x 30 x 20 cm) filled with 10 l of UV-irradiated, filtered seawater. Gametes were obtained following the methods of Chen and Yang (1979) by artificially inducing spawning with ultraviolet (UV) light and thermal violence. Water temperature was gradually increased with a heater from 23° to 28°C over 5 hr (mean rate of increase 1°C/hr) and then gradually decreased with 4°C seawater from 28° to 23°C over 2.5 hr (mean rate of decrease 2°C/hr). Two or three such cycles were sufficient to induce male and female spawning. The water temperature was maintained at 24° ± 1°C during spawning (Yang and Chen, 1979).

Investigation of polar body release time

Eggs (ca. 200 μm in diameter) were collected on a 60-μm mesh screen. The unfertilized eggs were divided into groups of 10^4 individuals and each group was suspended in a 1 liter beaker of seawater at 19°, 21°, 24°, 27°, or 30°C, respectively. Sperm solution (2 x 10^5 per ml) was also divided into five parts (10 ml/part) and each was placed in a 50 ml beaker at one of the five above-mentioned temperatures. For insemination, 1 ml of the stock sperm solution was added to the egg solution at the same corresponding temperature. After insemination, a sample of eggs (10^2) was drawn every 2-3 min and fixed in 5% formalin. The time of the release of the first and second polar bodies, as well as the first cleavage of zygotes, was defined as the time when 50% of the eggs in a sample had reached that stage.

Cold shock treatment

3 min after insemination at 24°C (to inhibit the release of PB1), four samples of inseminated eggs (10^4) were collected on a 60-μm mesh screen and placed into 3°C cold shock treatment for 5, 10, 20, and 40 min, respectively. Four additional samples were collected at 10 min post-insemination (to inhibit the release of PB2), and treated in the same cold shock series. After treatment, each egg sample was placed into a 2 l beaker of seawater at 24°C at a density of 5 eggs/ml. The egg suspensions were not aerated and were maintained in fresh seawater by decanting and replacing the upper two-thirds of water every hour until the eggs had settled to the bottom of the beaker.

Determination of percent normal embryos

Morphologically normal embryos have been shown to have a higher survival rate in the subsequent floating stages. Following the technique of Yang and Chen (1979) for calculating the percentage of normal gastrulae to estimate the effect of the length of time in cold shock treatment, samples of embryos (10^2) were collected 6 h after insemination and fixed in 5% formalin. Normal embryos at this stage have a smooth surface with a circular shape. In abnormal embryos, the cells are damaged, are of different sizes, and their shape is not circular.

Determination of ploidy

Chromosome squashes were prepared from gastrulae in each treatment using the methods of Allen *et al.* (1989). Ploidy was determined by counting chromosomes. Categories of ploidy were: haploid (1n = 15-16), diploid (2n = 31-32), triploid (3n = 47-48), tetraploid (4n = 63-64), and pentaploid (5n = 79-80). All others were considered aneuploid. Photographs were taken with Kodak Technical Pan Film 2415. Karyotype analyses were performed as described by Arai *et al.* (1986).

Data analysis

Analysis of Variance (ANOVA) and Duncanís analysis (Sokal and Rohlf, 1981; Statsoft, 1993) were used to determine the significance of differences between the control and treatments in terms of percent normal embryos and percent diploidy, triploidy, and aneuploidy.

RESULTS

Normal meiosis after insemination releases the first polar body (PB1) and second polar body (PB2), after which the egg undergoes the first cleavage (Fig. 1). In this study, temperature influenced the time of polar body release and cleavage of the egg. The higher the temperature, the shorter the time before polar body release and first cleavage (Fig. 2). At 24°C, PB1 was released at *ca.* 8 min and PB2 at *ca.* 19 min after insemination; the first egg cleavage occurred at *ca.* 43 min. These cytological events took less than half as long to occur in eggs maintained at 19°C than at 30°C.

Cold shock effectively inhibited polar body release. Following insemination, treated eggs released only one polar body after anaphase II while untreated eggs undergoing normal meiosis released both PB1 and PB2 (Fig. 3).

From microscopic examination of the embryos, 68 ± 2.3% of the control group exhibited normal gastrulae. In the PB1 groups, 21 ± 3.0%, 17 ± 3.5%, and 7 ± 2.0% of the gastrulae were normal after treatment times of 5, 10, and 20 min, respectively, while for the PB2 groups, 12 ± 2.6%, 15 ± 2.6%, and 3 ± 2.5% were normal. All eggs subjected to 40 min cold shock at the PB1 or PB2 stage produced deformed embryos by the gastrula stage. When comparing percent normal embryos in the treatment and control groups, all treatments of PB1 and PB2 groups differed significantly ($p < 0.001$) from the control. Between PB1 and PB2 groups, however, there were no significant differences ($p > 0.05$) for each of the treatment times.

Once an egg completes meiosis I and II, the chromosomes of the male nucleus combine with those of the female nucleus. This is followed by mitosis and subsequent development. In our observations of the chromosomes in cells at the gastrula stage, the control and treatment groups exhibited six types of ploidy (Fig. 4; Table 1). In the control group, 41.8% of the embryos were diploid, 9.0% haploid, 7.5% triploid, and 41.7% aneuploid. No tetraploids or pentaploids were found in the control.

Variations in ploidy in relation to treatment time and in polar body between treatment and control groups were

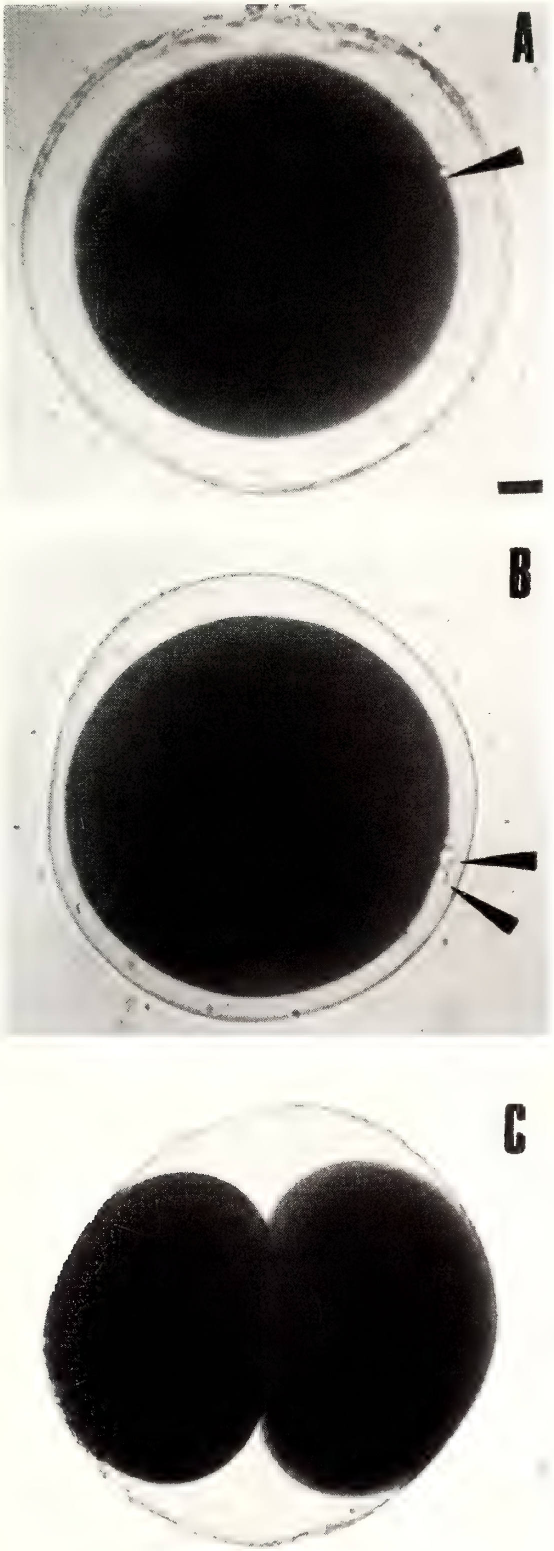

Fig. 1. Inseminated egg of *Haliotis diversicolor* undergoing normal meiosis. A. First polar body (PB1) released after anaphase I of meiosis I. B. Second polar body (PB2) released after anaphase II of meiosis II. C. First cleavage of the egg. Scale bar = 20 µm.

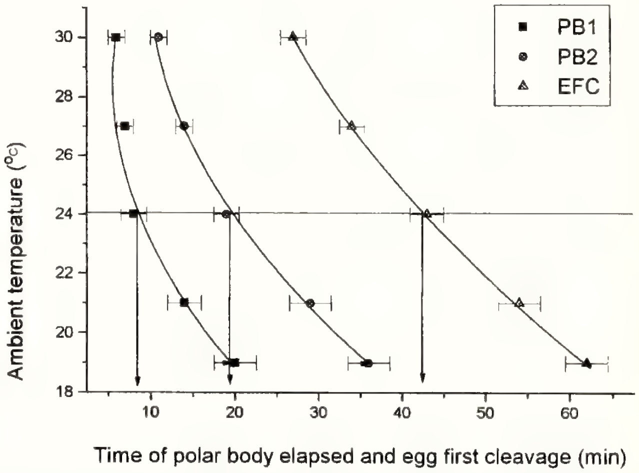

Fig. 2. Time of first polar body (PB1) and second polar body (PB2) release and first cleavage of the egg (EFC) in *Haliotis diversicolor*, as observed after insemination at five water temperatures.

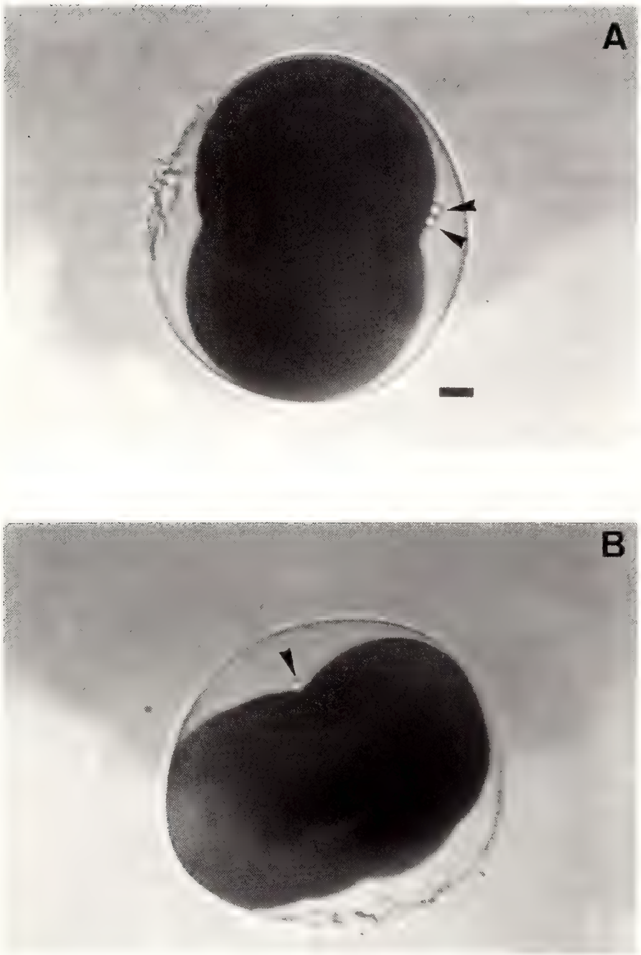

Fig. 3. Inseminated egg of *Haliotis diversicolor* after anaphase II of meiosis II. A. Without cold shock treatment; two polar bodies observed (arrows). B. After cold shock treatment; only one polar body (arrow) observed. Scale bar = 20 μm.

analyzed by ANOVA. There were significant differences in the diploid category for immersion time ($F_{4,4} = 6.88$, $p < 0.05$) and polar body ($F_{2,6} = 8.27$, $p < 0.05$). Duncan's analysis of the factors of time and polar body in relation to diploidy showed that 10 min of immersion time differed significantly ($p < 0.05$) from 5, 20, and 40 min, and that there were significant differences between PB1 and PB1 with the control, but no significance ($p > 0.05$) between PB1 and PB2.

Studies of the karyotypes of diploid, triploid, and tetraploid small abalone revealed that eight chromosomes are metacentric, seven are submetacentric, and one is subtelocentric/acrocentric (Fig. 5). The distribution of chromosome numbers in control and treated groups is shown in Fig. 6. In the control group, as expected, most embryos were normal diploids with 32 chromosomes, while in all the treated groups, most embryos were aneuploid, *i. e.* $2n \neq 32$. In the treated groups, only a small percentage of triploid and tetraploid embryos were found, most embryos being aneuploid.

DISCUSSION

Species and the environmental nature of the habitat affect the release time of the first and second polar bodies. In the Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), ten maternal tetrad chromosomes undergo meiosis I and II, and release two polar bodies (Arai *et al.*, 1986; Quillet and Panelay, 1986; Guo *et al.*, 1992; Longo *et al.*, 1993). The release times of PB1 and PB2 are 17 and 35 min, respectively, at 24-25°C (Allen *et al.*, 1989); these and the time of the first egg cleavage are influenced by water temperature (Arai *et al.*, 1986; Kudo *et al.*, 1991). In the small abalone studied here, the release times of PB1 and PB2 were 8 and 19 min, respectively. *Haliotis discus hannai* from northern Japan has release times of 15 and 38 min, respectively, at 25°C (Arai *et al.*, 1986). In this report, variation in temperature was a major factor affecting polar body release and cell cleavage time.

In this study, the percentage of morphologically normal embryos was used as a measure of larval survival. Although "percent normal" is admittedly not equal to "percent survival," normal morphological development does have a positive relationship with survival. This study of the effect of cold shock on the percentage of normal gastrulae showed that temperature was the major factor causing damage to eggs; the longer the immersion time, the greater the percentage of deformed embryos. All four PB1 and PB2 groups showed a low percentage of normal gastrulae, but there were no differences between PB1 and PB2 groups ($p > 0.05$); confirmation of corresponding larval survival requires further studies. These results differ from those of Guo *et al.* (1992) who found that in the Pacific oyster, blocking PB1 always resulted in higher mortalities than did blocking PB2. The results published by Kudo *et al.* (1991) on *Haliotis diversicolor diversicolor* (*i. e.* survival rates of 57 and 16%, respectively, in PB1 and PB2 groups subjected to 10 min cold shock) are likewise contrary.

Treated groups exhibited lower percentages of diploids than the control. This could indicate that the cold shock treatment effectively inhibited the polar body of the egg. However, the treatments did not produce a higher percentage of triploids than diploids in the control, suggesting that chromosome segregation does not always lead to triploidization.

The occurrence of haploidy and triploidy in the control groups was not due to cold shock, suggesting that haploids and triploids occur spontaneously in small abalone. Spontaneous haploids could be caused by the failure of sperm pronucleus incorporation, while triploids have been

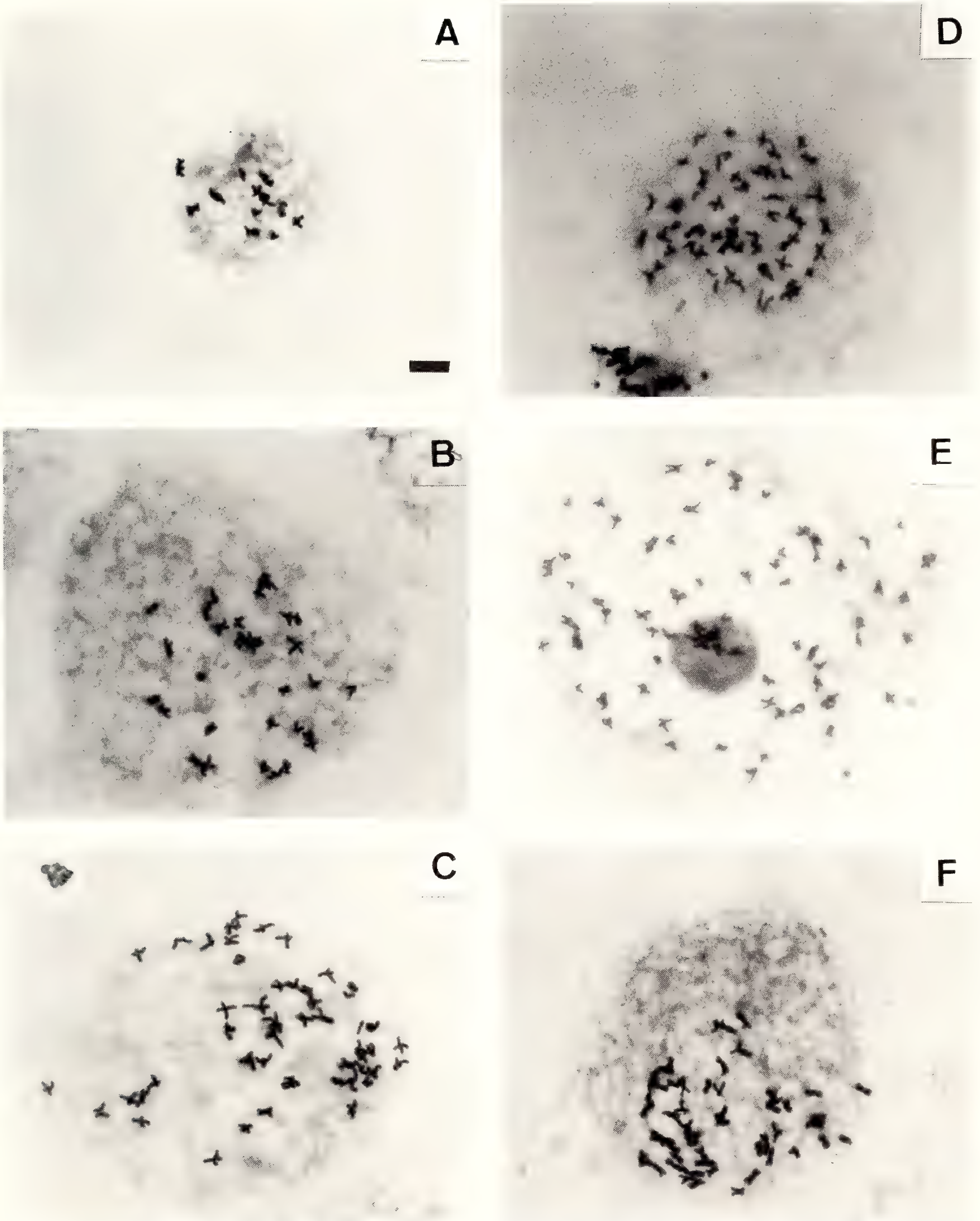

Fig. 4. Categories of ploidization of chromosomes in *Haliotis diversicolor* embryos treated by cold shock after insemination. A. Haploid. B. Diploid. C. Triploid. D. Tetraploid. E. Pentaploid. F. Aneuploid. Scale bar = 20 μm.

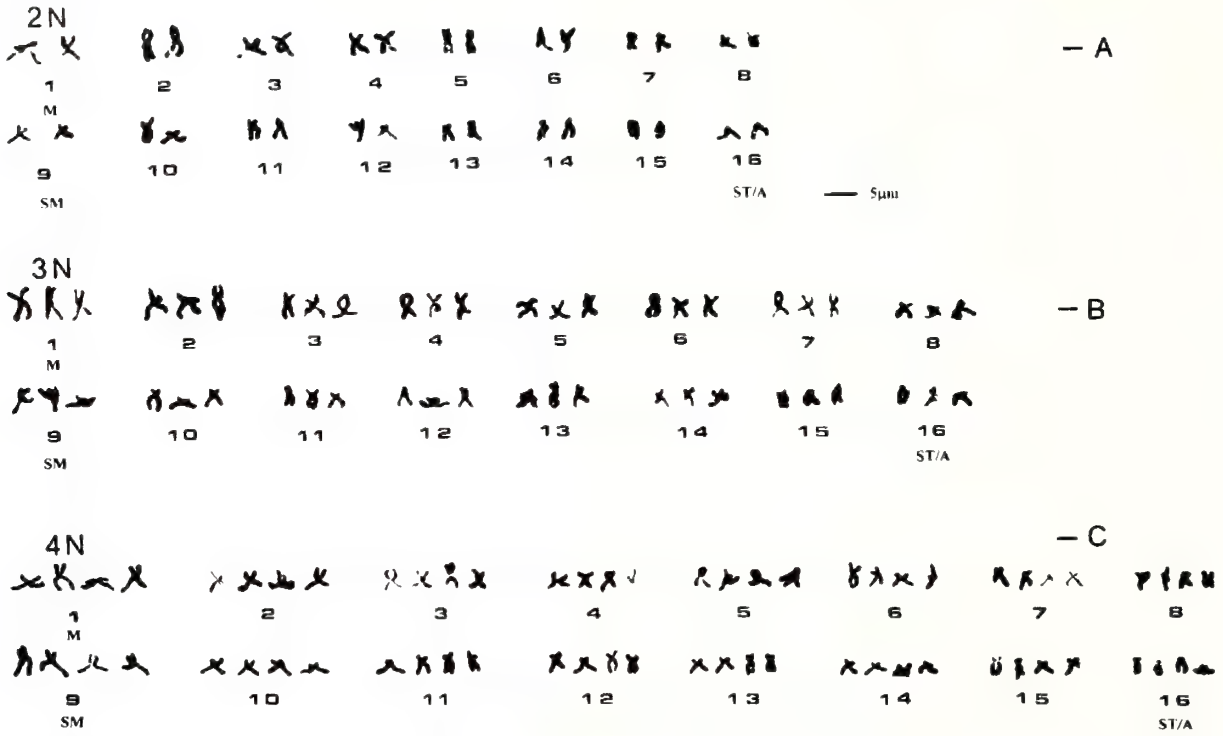

Fig. 5. Karyotypes of diploid (A), triploid (B), and tetraploid (C) *Haliotis diversicolor*. Pairs 1-8 are metacentric (M), 9-15 are submetacentric (SM), and pair 16 is subtelocentric/acrocentric (ST/A). Scale bar = 5 μm.

Table 1. Percentages of morphologically normal *Haliotis diversicolor* embryos (± standard error), and of the various ploidy levels (with number of observations in parentheses) in the control and various treatments (immersion times), following 3°C cold shock blocking the first polar body (PB1) or second polar body (PB2). (1n, haploid; 2n, diploid; 3n, triploid; 4n, tetraploid; 5n, pentaploid; An, aneuploid; *, $p < 0.05$; **, $p < 0.001$).

			% Ploidization						
Group	Polar body	% Normal	N	1n	2n	3n	4n	5n	An
Control		68 ± 2.3	67	9.0	41.8	7.5	0.0	0.0	41.7
		(153)		(6)	(28)	(5)			(28)
5 min	PB1	21 ± 3.0**	151	21.9	10.6	4.0	1.3	0.7	61.5
		(147)		(33)	(16)	(6)	(2)	(1)	(93)
5 min	PB2	12 ± 2.6**	19	26.3	15.8	5.3	0.0	0.0	52.6
		(104)		(5)	(3)	(1)			(10)
10 min	PB1	17 ± 3.5**	240	7.5	16.7*	10.0	3.8	1.7	60.3
		(187)		(18)	(40)	(24)	(9)	(4)	(145)
10min	PB2	15 ± 2.6**	91	8.8	20.9*	5.5	1.1	0.0	63.7
		(147)		(8)	(19)	(5)	(1)		(58)
20 min	PB1	7 ± 2.0**	283	7.1	16.3	11.0	1.8	0.4	63.4
		(164)		(20)	(46)	(31)	(5)	(1)	(180)
20 min	PB2	3 ± 2.5**	15	0.0	13.3	6.7	6.7	0.0	73.3
		(164)			(2)	(1)	(1)		(11)
40 min	PB1	0	100	26.0	14.0	5.0	4.0	2.0	49.0
		(179)		(26)	(14)	(5)	(4)	(2)	(49)
40 min	PB2	0	13	38.5	0.0	0.0	0.0	0.0	61.5
		(168)		(5)					(8)

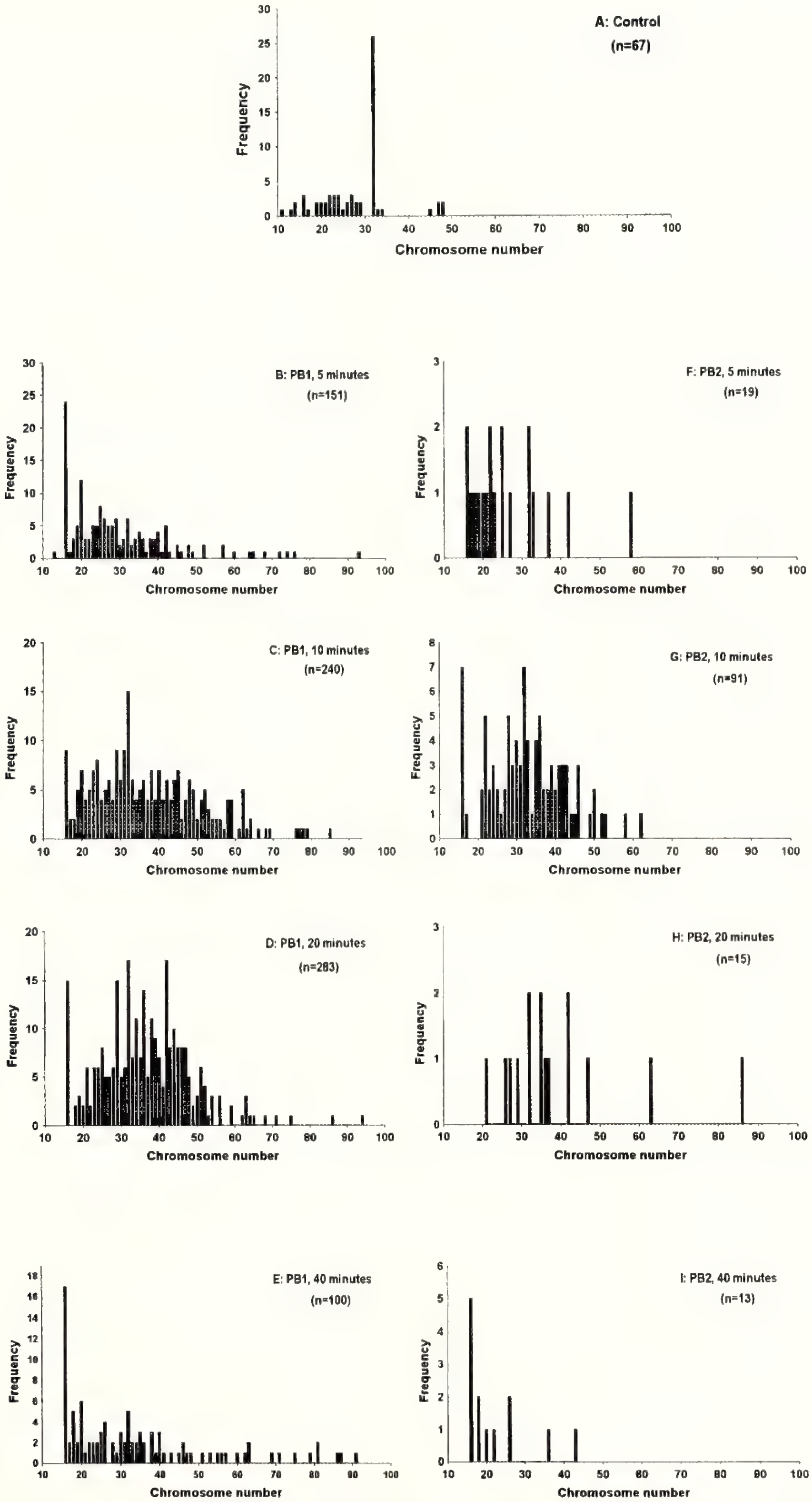

Fig. 6. Distributions of chromosome counts in the control and treatment groups of *Haliotis diversicolor* embryos treated with 3°C cold shock blocking the first polar body (PB1) or second polar body (PB2). Untreated control (A). Treatment of PB1 groups: 5 min (B), 10 min (C), 20 min (D), 40 min (E). Treatment of PB2 groups: 5 min (F), 10 min (G), 20 min (H), 40 min (I). (n, numbers of embryos).

reported in amphibians and fish and are probably caused by fertilization of unreduced ova (Fankhauser, 1945; Thorgaard and Gall, 1979).

In this study of small abalone, 41.7% aneuploids were observed in the control. Aneuploids have commonly been assumed to be artifacts caused by poor karyological techniques such as chromosome loss or overestimation. In this study, a statistical significance between the control and treated groups ($p > 0.05$) demonstrated that aneuploids were not a consequence of cold shock treatment. The formation of aneuploid gametes in mollusks can be common, particularly under conditions of environmental stress (Dixon, 1982). Guo *et al.* (1992) used the chemical cytochalasin B to inhibit PB1, and the resulting aneuploids in the various treatments were distributed around two peaks. The high level in small abalone is in contrast to a low proportion (9%) in the Pacific oyster (Guo *et al.*, 1992), perhaps indicating that the abalone is more sensitive to the environment. Aneuploidy often leads to abnormality and death in higher animals (Dixon, 1982). In studies where ploidy was determined at juvenile or adult stages (Stanley *et al.*, 1981; Downing and Allen, 1987), aneuploids could have been absent because they had already died during early embryonic development. The high percentage of aneuploidy in small abalone suggests that blocking the polar bodies caused disruption of normal chromosome segregation, and could explain the higher mortality rate at the settling stage (90%) experienced during artificial propagation (Yang and Ting, 1988).

The results of this study regarding triploids and tetraploids inhibiting PB1 or PB2 agree with those for the Pacific oyster (*Crassostrea gigas*; Guo *et al.*, 1992), the American oyster [*C. virginica* (Gmelin, 1791); Stanley *et al.*, 1981], the mussel [*Mytilus edulis* (Linné, 1758); Yamamoto and Sugawara, 1988], and the Pacific abalone (*Haliotis discus hannai*; Arai *et al.*, 1986). On the other hand, results of this study differ from those of other studies in which only triploid embryos (Quillet and Panilay, 1986) or only tetraploid embryos (Stephens, 1989) were observed to inhibit PB1. This difference could be due to different experimental methods and conditions. Comparison of treated groups and controls in the diploid category were a consequence of cold shock at 10 min. Variation in these environmental factors was probably also responsible for the different proportions of ploidy observed in the immersion times and between the two polar bodies in this study.

The percentage of normal gastrulae and production of triploids showed that the optimal cold shock immersion time is between 5 and 10 min. The production of various levels of ploidy by inhibiting PB1 and PB2 demonstrated the complex nature of chromosome segregation. More rigid inducing conditions and the mechanism of chromosome segregation will be investigated in future studies.

ACKNOWLEDGMENTS

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The gametogenic cycle of *Brachidontes exustus* (Linné, 1758) (Bivalvia: Mytilidae) at Wassaw Island, Georgia

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Abstract: The gametogenic cycle of a population of *Brachidontes exustus* (Linné, 1758) was studied from January to December 1993 at the northern beach of Wassaw Island, Georgia. Staging values were assigned for Gonadal Indexing (GI) of histologically prepared gonadal tissue: 1 = spent; 2 = partially spawned; 3 = early active; 4 = late active; 5 = ripe. From January to March and September to December, males had significantly higher (ANOVA, $p = 0.0249$) GI values when compared to females. Because male and female GI showed no significant difference (ANOVA, $p = 0.1244$) over the major reproductive period (April to August), they were averaged per sample period. Rapid gonadal development occurred from March to mid-May and follicles were ripe by mid-July. A minor spawn might have occurred in late July. A major spawn occurred from September to November. Of 261 animals sampled, the sex ratio was significantly different (Chi-square = 7.48; $p < 0.05$) from parity (1.00 female:1.43 male) and 9.6% were sexually undifferentiated. The greatest percentage of sexually undifferentiated individuals occurred in March (66.7%) with fewer from January to April. No hermaphrodites were observed.

Key words: *Brachidontes*, gametogenesis, mussel, reproduction, sex ratio

Brachidontes exustus (Linné, 1758), commonly inhabits rock pilings, sea walls, and wharf pilings in the intertidal zone and is most abundant in lower intertidal regions (Seed, 1980). The mussel ranges in the United States from North Carolina to Texas (Abbott, 1974). Information concerning species of *Brachidontes* is limited. Studies include life habitats (Seed, 1980), surface and shell morphology (Fuller and Lutz, 1988, 1989), and larval development (Campos and Ramorino, 1980; Fields and Moore, 1983). In addition, Morton (1988) studied the reproductive cycle of *B. variabilis* (Krauss, 1848) in a Hong Kong mangrove and showed that juveniles grew rapidly, are sexually mature at one-year of age, and had a short life span (< 3 years). Gametogenesis in *B. variabilis* was initiated in winter with individuals spawning in summer and being spent by October-November (Morton, 1988).

Gametogenic cycles have been studied in other marine mussels including *Geukensia demissa* (Dillwyn, 1817) (Heffernan and Walker, 1989; Borrero, 1987; Brousseau, 1982); *Modiolus capax* (Conrad, 1937) (Aguirre and Ramirez, 1989); *Modiolus metcalfei* (Hanley, 1843) (Lopez and Gomez, 1982); *Modiolus modiolus* (Linné, 1758) (Seed and Brown, 1977; Jasim and Brand, 1989); *Modiolus philippinarum* (Hanley, 1843) (Walter and Cruz, 1980); *Mytilus edulis* (Linné, 1758) (Seed and Brown, 1977; Brousseau, 1983); and *Perna picta* (Born, 1778) (Shafee, 1989). Gametogenic studies of species in the southeastern U. S. include comparisons of *G. demissa* populations in South Carolina (Borrero, 1987), an energy flow study of a *G. demissa* population at Sapelo Island, Georgia (Kuenzler, 1961), and a study of reproduction in *G. demissa* at Wassaw Sound, Georgia (Heffernan and Walker, 1989).

There is little information on the life history of *Brachidontes exustus* and no gametogenic study. The purpose of this study was to determine the gametogenic cycle of a population of *B. exustus* at Wassaw Island, Georgia, through histological examination.

METHODS

Between 15 and 20 individuals of *Brachidontes exustus* (1-2 cm in length) were collected monthly from January to March 1993, biweekly from April to September 1993, and monthly from November to December 1993, from the northern beach of Wassaw Island, Georgia (Fig. 1). Inclement weather prevented an October 1993 sampling. Individuals occurred at the base of dead oak trees on the shoreward side within the intertidal surf zone.

Shell length (distance anterior-to-posterior) was

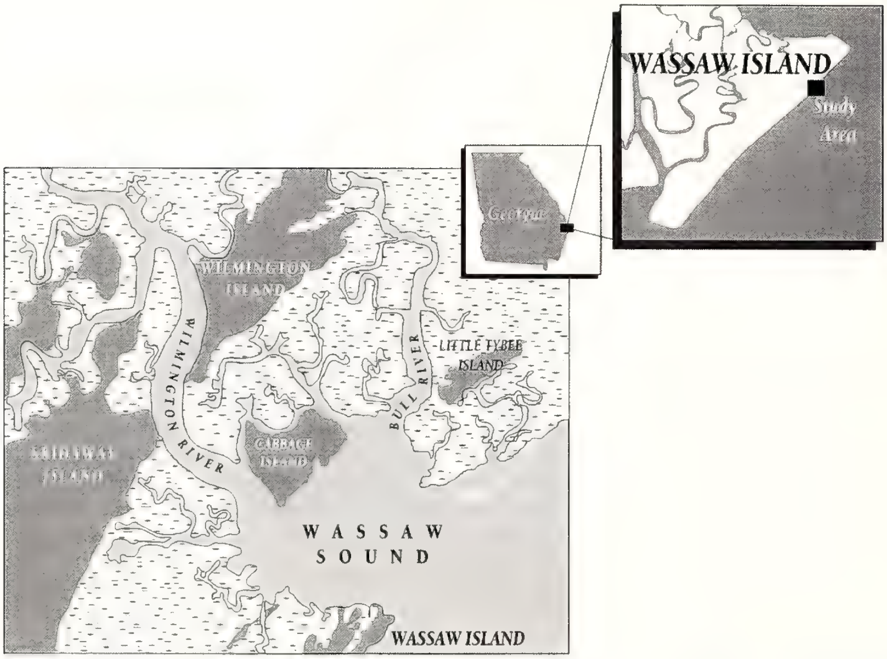

Fig. 1. Location of *Brachidontes exustus* population occurring at the base of dead oak trees in the intertidal surf zone on Wassaw Island, Georgia.

determined using Vernier calipers. The visceral mass was fixed in Davidson's solution (Humason, 1967) for 48 hr, rinsed in 50% alcohol overnight, and stored in 70% alcohol. Tissue samples were dehydrated in a graded alcohol series, cleared in toluene, and embedded in Paraplast®. Paraffin blocks were sectioned (7-8 µm) with a Leica 820 II Microtome. The tissue sections were deparaffinized with toluene, rehydrated to water, and then stained with Harris, Hematoxylin and counterstained with Eosin (Bancroft and Stevens, 1982). Slides were examined and photographed under a light microscope.

Gonadal tissue was rated according to characteristics of gametes and stage of maturity, as described for other species (Brousseau, 1982, 1983; Heffernan and Walker, 1989). Gonadal tissue also occurred in the mantle of all individuals. The gametogenic cycle was divided into the following categories:

Sexually Undeveloped = 0. No male or female gametes detected. Follicles small and shrunken with little or no lumen.

Male Developmental Stages:

Spent = 1. Follicles empty with a large lumen space. A few spermatozoan cells scattered throughout the follicle. Gonads appear much smaller than those in ripe individuals. Blood cells present in the follicles (Fig. 2a).

Partially spawned = 2. Most follicles with the clear lumen surrounded by clusters of spermatozoa (Fig. 2c).

Early active = 3. A narrow-to-wide band of large cells (spermatogonia and/or spermatocytes) located inside each testis follicle. No smaller spermatids or spermatozoa cells present. Follicle lumen large and devoid of cells (Fig. 2e).

Late active = 4. A wide band of larger cells (spermatogonia and spermatocytes) present along the inside of the testis follicle. Lumen small to nonexistent. Some follicles with small pink streaks. Smaller spermatozoa present toward the center of the follicle (Fig. 2g).

Ripe = 5. Gonads full of spermatocytes, spermatids, and spermatozoa, making it difficult to differentiate cell types. Follicles stained black to dark purple with pink streaks in the center (Fig. 2i).

Female Developmental Stages:

Spent = 1. Follicles mostly empty with only a few scattered oocytes. Blood cells present; follicles smaller than when ripe (Fig. 2b).

Partially spawned = 2. Most of the oocytes mature, each with a large nucleus; many eggs unattached to the follicle wall. Lumen space large. Many follicles with a few or no mature oocytes (Fig. 2d).

Early active = 3. Follicle lumen devoid of cells. Young oocytes with small nuclei attached to the follicle wall (Fig. 2f).

Late active = 4. Oocytes large, elongate, some attached to the follicle wall. Follicles with several mature oocytes present but lumen incompletely filled with eggs (Fig. 2h).

Ripe = 5. Many large mature oocytes present; in most specimens the lumen space is filled. A few oocytes

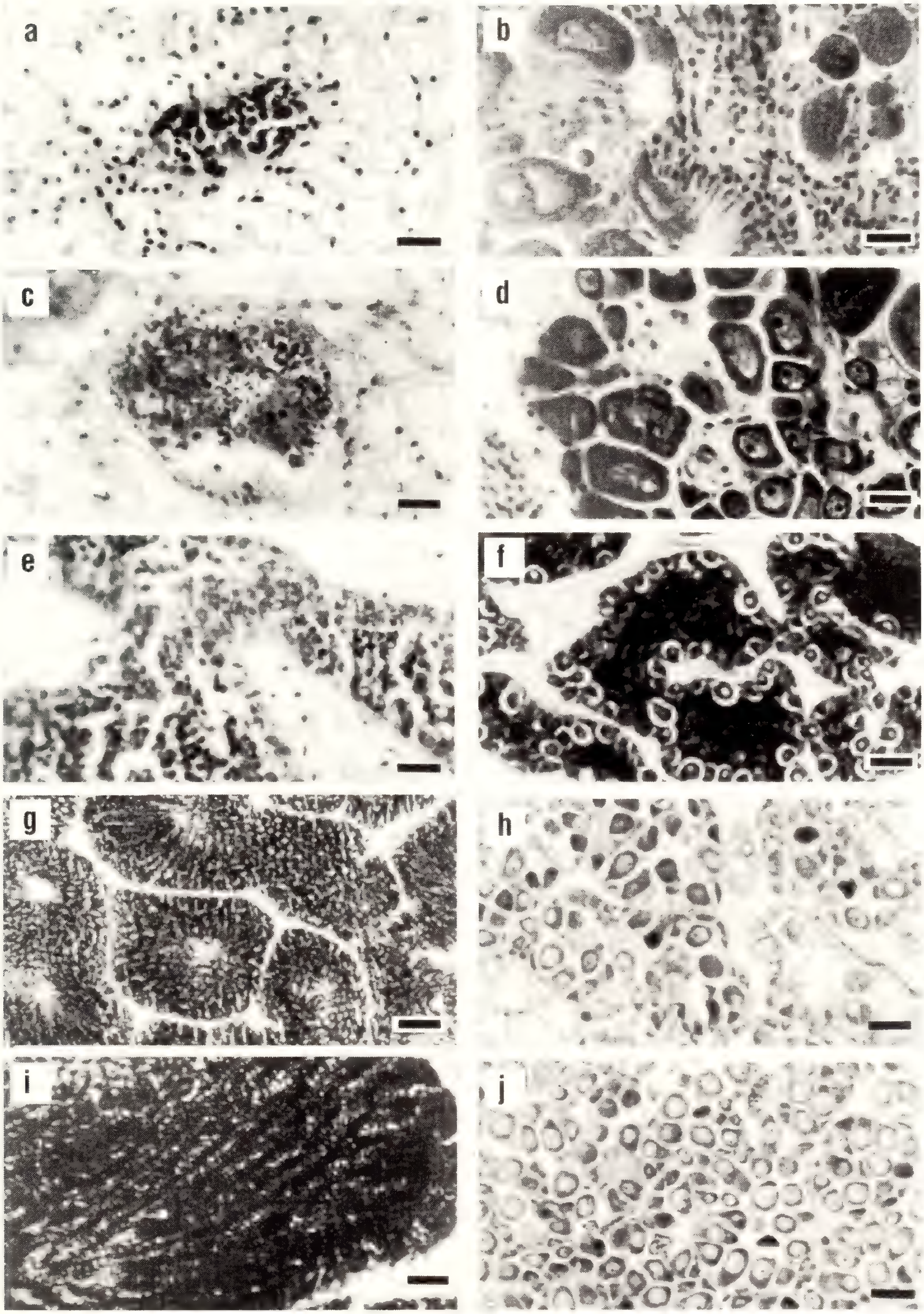

Fig. 2. Reproductive stages in male and female *Brachidontes exustus*: a. Spent male. b. Spent female. c. Partially spawned male. d. Partially spawned female. e. Early active male. f. Early active female. g. Late active male. h. Late active female. I. Ripe male. j. Ripe female. Scale bars = 20 μm (a, b, c, d, e, g, i) or 50 μm (f, h, j).

attached to follicle wall (Fig. 2j).

The sex of each individual was determined and the sex ratio of the population (biweekly sample size, N = 15) was tested for non-parity by Chi Square analyses (Elliott, 1977). Statistical analyses of male and female gonadal index values were analyzed by Analysis of Variance (ANOVA) ($\alpha = 0.05$) using SAS for PC computer (SAS Institute, 1989). T-tests ($\alpha = 0.05$) (SAS Institute, 1989) were performed on gonadal index values and shell length data to determine if significant differences occurred at any specific time period.

RESULTS

Individuals varied in shell length from 11.3 to 24.6 mm (mean = 17.3 ± 0.15 mm SE; N = 261). A total of 261 mussels were sexed with 9.6% sexually undifferentiated, 37.2% female, and 53.2% male. Sex ratio was significantly different from parity (Chi-square = 7.48; $p < 0.05$) with a ratio of 1.00:1.43 female to male.

Female and male gonadal indices are presented in Fig. 3. ANOVA revealed that males had significantly ($p = 0.0249$) higher GI values over time than females. Males had significantly higher GI values, as determined by T-tests from January to March and from September to December. No significant difference (ANOVA; $p = 0.1244$) in GI value occurred between males and females from April to August. Males had a longer gametogenic cycle than females. Females showed a more rapid drop in GI value (N = 7, mean = 4.86 ± 0.14; N = 6, mean = 1.83 ± 0.65) over males (N = 8, mean = 5.00 ± 0.00; N = 9, mean = 3.67 ± 0.53) during peak spawning (August-September).

Gonadal development was evident throughout most of the year, except March. The lowest mean GI value for males, females, and sexually undifferentiated individuals combined occurred in March (N = 15, mean = 0.58 ± 0.29; Fig. 4), during which the highest percentage of undifferentiated animals (66.7%) occurred. The mean GI for males and females combined increased from March (N = 15, mean = 0.58 ± 0.29) to April (N = 15, mean = 2.27 ± 0.38) and reached a peak in mid-July (N = 15, mean = 4.79 ± 0.29). By mid-July, 92.9% of the population was ripe and 7.1% was partially spawned. Low percentages of individuals in the partially spawned stage occurred from May to July (Fig. 4), which could be representative of dribble spawning, *i. e.* when males and females continually produce and release sperm and egg cells, respectively. Indications of spawning were not reflected in mean GI values (Figs. 3-4) during May to July. The combined mean GI value for males and females increased to 4.31 in mid-August, with 76.9% ripe individuals and 23.1% at the partially spawned stage. The highest GI value (N = 15, mean = 4.93 ± 0.07) was reached in late August when 93.3% of the sampled population was ripe and 6.7% were spent. A major spawn commenced in early September when there was a decrease in mature gametes (N = 15, mean GI = 4.25 ± 0.39). Spawning continued until December (N = 21, mean GI = 1.95 ± 0.43) when 40% was spent, 15% was partially spawned, 20% was undifferentiated, and 25% was at the ripe stage (Figs. 4-5).

DISCUSSION

The gametogenic cycle of *Brachidontes exustus* from a population in coastal Georgia showed a unimodal

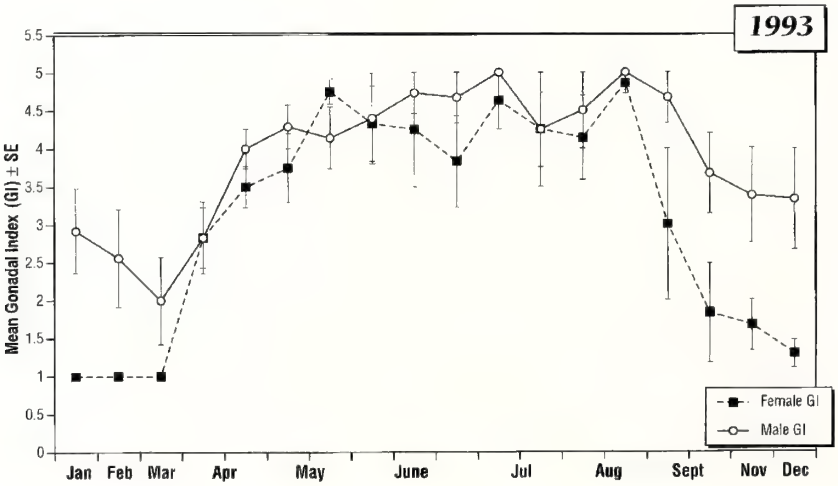

Fig. 3. Mean monthly gonadal index values ± SE for male and female *Brachidontes exustus* collected at Wassaw Island, Georgia.

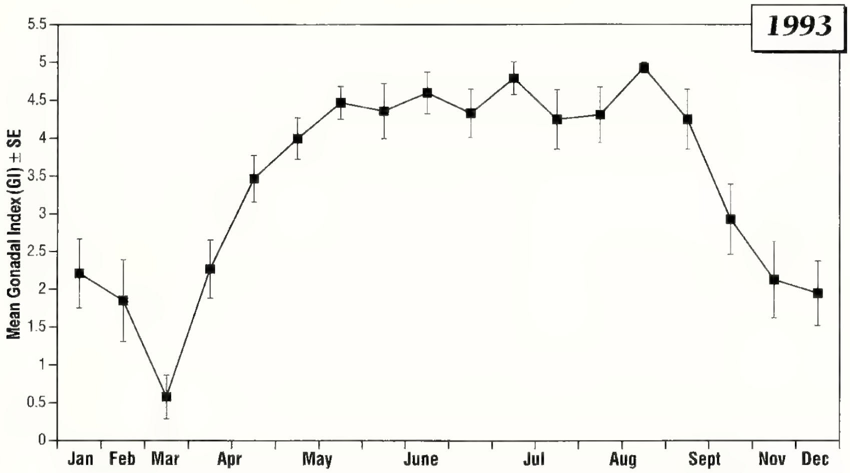

Fig. 4. Mean monthly mean gonadal index values combined for male and female *Brachidontes exustus* collected at Wassaw Island, Georgia.

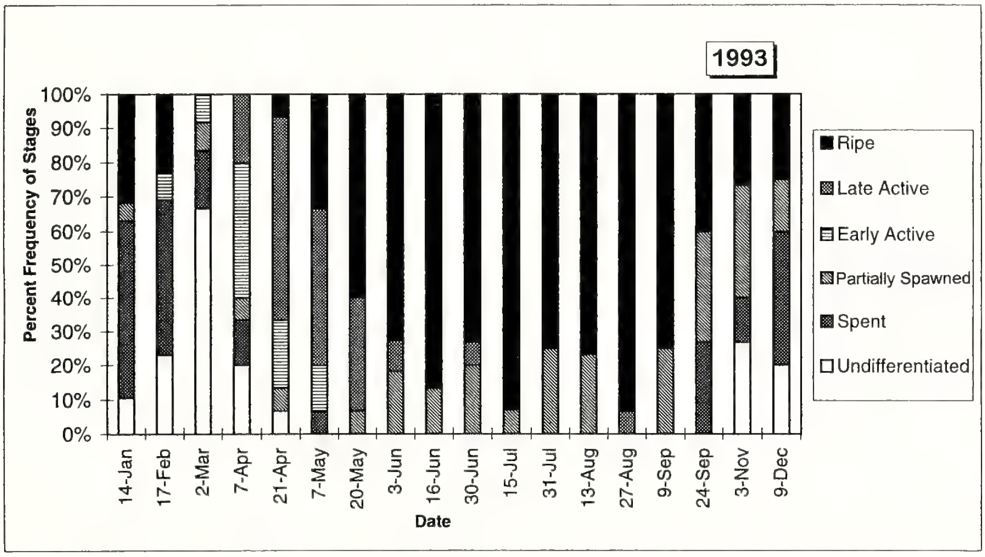

Fig. 5. Relative frequency of gonadal stages over time in *Brachidontes exustus* collected at Wassaw Island, Georgia.

pattern (Figs. 3-4) during 1993, when gamete production increased from March until July, with a major spawn occurring in August and September. Dribble spawning occurred from May to August (Fig. 4) and this phenomenon was similar to the spawning pattern observed in *Mytilus edulis* (see Brousseau, 1983). Brousseau (1983) attributed dribble spawning as a response to variations in environmental conditions over a period of time. The spawning period (August-September 1993) of *B. exustus* at Wassaw Island showed similarities to a *Geukensia demissa* population in Georgia, which spawned from August to October 1984 and July to September 1985 (Heffernan and Walker, 1989). The results of the present study were also similar to Kuenzler's (1961) results (spawning July-September) for a population of *G. demissa* on Sapelo Island, Georgia, and to those of a study of a population of *G. demissa* at North Inlet Estuary, South Carolina (Borrero, 1987). Borrero (1987) showed that individuals from low intertidal areas increased in gonadal development from May to early August, with a minor spawn occurring in June and a major spawn from August through September. However, a longer spawning period, resembling our results, occurred in a Hong Kong mangrove population of *B. variabilis* (see Morton, 1988). Morton (1988) found that the population of *B. variabilis* ripened in the summer and by November and December 1985, the population was spent.

The lowest mean gonadal index value in March (0.58 ± 0.29) for *Brachidontes exustus* was followed by an increase throughout the spring until peak values were attained in July (mean = 4.79 ± 0.21). The increase in

Table 1. Habitat, sex ratio, and percent hermaphrodites of various mytilid mussels. (E, estuarine; D, deep sea; M, marine; ND, not determined).

Species	Habitat	N	Female/Male	% Herm.	Source
Brachidontes exustus	M	261	1.00:1.43*	0	This Study
B. variabilis	E	247	0.80:1.00	ND	Hulings, 1986
B. variabilis	E	330	1.00:0.69*	0	Morton, 1988
Choromytilus meridionalis (Krauss, 1848)	M	700	0.75:1.00*	0	Griffiths, 1977
Crenomytilus grayanus (Dunker, 1882)	ND	ND	1.00:1.00	ND	Dolgov, 1985
Geukensia demissa	E	354	1.00:1.00	ND	Brousseau ,1982
G. demissa	E	429	1.00:1.00	0	Borrero, 1987
G. demissa	E	498	1.00:1.00	0	Heffernan and Walker, 1989
Idas argenteus (Jeffreys,1876)	D	101	1.00:0.83	0	Dean, 1993
Lithophaga bisulcata (d'Orbigny, 1842)	M	157	1.00:1.00	ND	Scott, 1988
		96	1.00:1.00	ND	
L. nigra (d'Orbigny, 1842)	ND	231	1.00:0.91	ND	Barkati and Asif, 1984
Modiolus barbatus (Linné, 1758)	ND	975	1.00:1.41	ND	Cahour and Lucas, 1968
		1060	1.00:1.66	ND	
M. metcalfei	E	1280	1.00:1.00	0.16	Lopez and Gomez, 1982
M. modiolus	M	5965	1.00:1.00	**	Jasim and Brand, 1989
M. philippinarum	ND	848	1.00:1.00	ND	Walter and Cruz, 1980
Mytella guyanensis (Lamarck, 1819)	ND	388	3.47:1.00*	ND	Sibaja, 1986
Mytilus edulis	ND	ND	1.00:1.18	ND	Coe, 1943
M. edulis	M	14,661	1.00:1.13*	ND	Seed, 1969
M. edulis	M	534	1.00:1.00	0.02	Brousseau, 1983
M. edulis	ND	3000	1.00:1.00	ND	Dolgov, 1985
M. edulis	ND	450	1.00:0.90	0	Sprung, 1983
M. edulis	M	346	1.00:0.79	0.3	Kautsky, 1982
M. galloprovincialis (Lamarck, 1819)	ND	ND	1.00:1.00	ND	Kudinskii and Shurova, 1990
Perna perna (Linné, 1758)	ND	275	1.00:1.00	0	Lasiak, 1986
P. picta	ND	800	1.00:1.20*	ND	Shafee, 1989
P. viridis (Linné, 1758)	M		1.00:1.00	0	Barkati and Ahmed, 1974
		648	1.00:0.98	0	
			1.00:0.79	0	
P. viridis	ND	1000	1.00:1.00	0	Walter, 1982
P. viridis	ND	1760	1.00:0.78	ND	Vakily *et al.*, 1988
P. viridis	ND	526	1.00:1.29	< 0.1	Lee, 1988
Perumytilus purpuratus (Lamarck, 1819)	ND	797	1.00:1.00	ND	Lozada and Reyes, 1982
Septifer virgatus (Wiesmann, 1837)	ND	382	1.00:0.78	0	Morton, 1995
Xenostrobus securis (Lamarck, 1819)	E	354	1.00:0.91	ND	Wilson, 1969
		316	1.00:0.84	ND	

* Reported to be significantly different from a 1:1 ratio by a Chi-Square test.

** Percent hermaphrodite not determined, but stated in text that some hermaphrodites were observed.

gonadal development during spring reflects the pattern of gametogenesis reported for *Geukensia demissa* populations in Georgia by Heffernan and Walker (1989). The highest percentage of sexually undifferentiated *B. exustus* (66.7%) occurred in March. Sexually undifferentiated mussels occurred in lower percentages during January (10.5%), February (23.1%), April (20.0%), November (26.7%), and December (20.0%) (Fig. 5). Heffernan and Walker (1989) found that during February 1984, 50.1% of the *G. demissa* sampled were sexually undifferentiated. Also, a higher percentage of undifferentiated *G. demissa* occurred in the winter months (November 90% and December 60%), followed by decreasing proportions of undifferentiated animals, and an increase in sexually developing individuals throughout the spring (Heffernan and Walker, 1989).

Within Mytilidae, sex ratios vary substantially (Table 1). In this study, *Brachidontes exustus* had significantly fewer females than males (1.00:1.43). For *B. variabilis*, Hulings (1986) found that significantly more males occurred in a population in the Gulf of Aqaba (Red Sea), while Morton (1988) found greater number of females than males in a Hong Kong estuarine population.

As with most members of the Mytilidae, *Brachidontes exustus* is dioecious and exhibits stable gonochronism. No hermaphroditic *B. exustus* were found. Hulings (1986) and Morton (1988) observed no hermaphrodites for *B. variabilis*. Within the Mytilidae, hermaphroditism is a rare event (Table 1).

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Aspects of gametogenesis of *Diplodon rotundus gratus* (Wagner, 1827) (Bivalvia: Hyriidae) in Brazil

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Abstract: The reproductive biology of the freshwater bivalve *Diplodon rotundus gratus* (Wagner, 1827) was studied in specimens collected from the Pardo River, in the northeastern region of the State of São Paulo, Brazil. Analysis of the gametogenic cycle revealed that the species is a functional hermaphrodite. The gametogenic cycle began at the end of spring (November) and continued until the beginning of winter (June). In spring and summer the population was found to consist of pure females with only a small proportion of hermaphroditic individuals. Active gametogenesis occurred between February and June. Mature oocytes and sperm occurred simultaneously in the fall. Spawning is probably affected by exogenous factors such as temperature and prolonged droughts associated with host biology. In winter, spring, and summer, no fish with glochidia were found, although glochidial release occurred from February to August.

Key words: Mollusca, Bivalvia, Hyriidae, gametogenesis, reproduction

Several reports of the reproductive processes in species of Unionoidea in North America and Europe are available (Arey, 1932; Ghosh and Ghose, 1972; Zumoff, 1973; Wood, 1974a, b; Heard, 1975; Nagabhushanam and Lohgaonker, 1978; Dartnall and Walkey, 1979; Chung, 1980; Kat, 1983). Particularly outstanding for the southern hemisphere are the studies by Peredo and Parada (1984, 1986) on *Diplodon chilensis chilensis* Gray, 1828, and by Jones *et al.* (1986) on Australian Hyriidae. However, few studies are available about the life cycle involving gametogenesis, time of incubation, glochidial elimination, host fish, and duration of parasitism for most freshwater bivalve species in southern regions. Systematic data and some biological data, especially concerning the larval forms of freshwater bivalves, have been reported by Ortmann (1921), Morretes (1949), Bonetto (1954, 1959, 1961a, b, 1962, 1964, 1965), Bonetto and Ezcurra (1963), Mansur (1970, 1973), Hebling and Penteado (1974), Hebling (1976), and Mansur and Anflor (1981).

It is important to understand the freshwater bivalve fauna of Brazil from many biological aspects, including their physiology, ecology, and systematic position. This is even more important for limnic bivalves that are poorly known and have been studied by few investigators. One such species is *Diplodon rotundus gratus* (Wagner, 1827), a species of wide geographic distribution on the South American continent (Bonetto, 1965). It is endemic to rivers of the Ribeirão Preto region (21°07'S, 47°45'W) where individuals live buried in muddy or sandy-muddy substratum quite close to the surface. Morretes (1949), Bonetto (1961a, 1965), Parodiz (1968), Mansur (1970), and Hebling and Penteado (1974) have provided data about its biology, systematics, larval forms, and functional anatomy. Like most unionid bivalves, *D. rotundus gratus* incubates embryos in its inner demibranchs where the embryos differentiate into glochidia after about one month, as observed in the South American fauna by Bonetto (1959, 1961a, b).

Our objective was to investigate the gametogenic cycle (development of gametes) and some aspects of the reproductive cycle (spawning and development) of *Diplodon rotundus gratus* to expand knowledge of its biology and to provide background information for future studies.

MATERIALS AND METHODS

Over a period of one year (February 1987 to January 1988) approximately 50 adult specimens were collected monthly from the Pardo River for histological study of the gametogenic cycle. The animals were located by probing the bottom of the river with the aid of feet and hands. They were fixed in aqueous Bouin's solution at the collection site, removed from their shells after 24 hr, preserved in 70% alcohol, then dehydrated, cleared, and embedded in paraffin for histology. Approximately 20 7-µm-thick transverse histological sections were obtained from each specimen (especially in the posterior region near the stomach where gonadal acini are more developed; Hebling and Penteado, 1974), mounted on microscope slides, and stained with Harris' hematoxylin and eosin, Mallory's trichrome, or combined stains (periodic-acid Schiff's + alcian blue + Harris' hematoxylin). The sections were analyzed under a light microscope for gametogenic phase and the various stages of follicular and marsupial development throughout the year.

From February 1991 to January 1992, approximately 50 adults were collected monthly for a complementary study of the reproductive cycle, again by probing the river bottom with feet and hands. The animals were transported alive to the laboratory in insulated boxes. The valves were pried open with a spatula to examine the general condition, development, and color of the marsupium. These macroscopic analyses were complemented by microscopic examination of a smear of the left inner demibranch of each animal. The observed stages of the marsupium were later compared to the microscopic analysis of the histological sections made earlier.

At the bivalve collection site, fish were captured with a net of 2-cm mesh. These were transported alive to the laboratory in insulated boxes, placed in aquaria, identified, and examined externally on the same day under a stereoscopic microscope to determine the presence of glochidia and/or lasids on the gills, fins, tail fins, and body.

RESULTS

GAMETOGENESIS

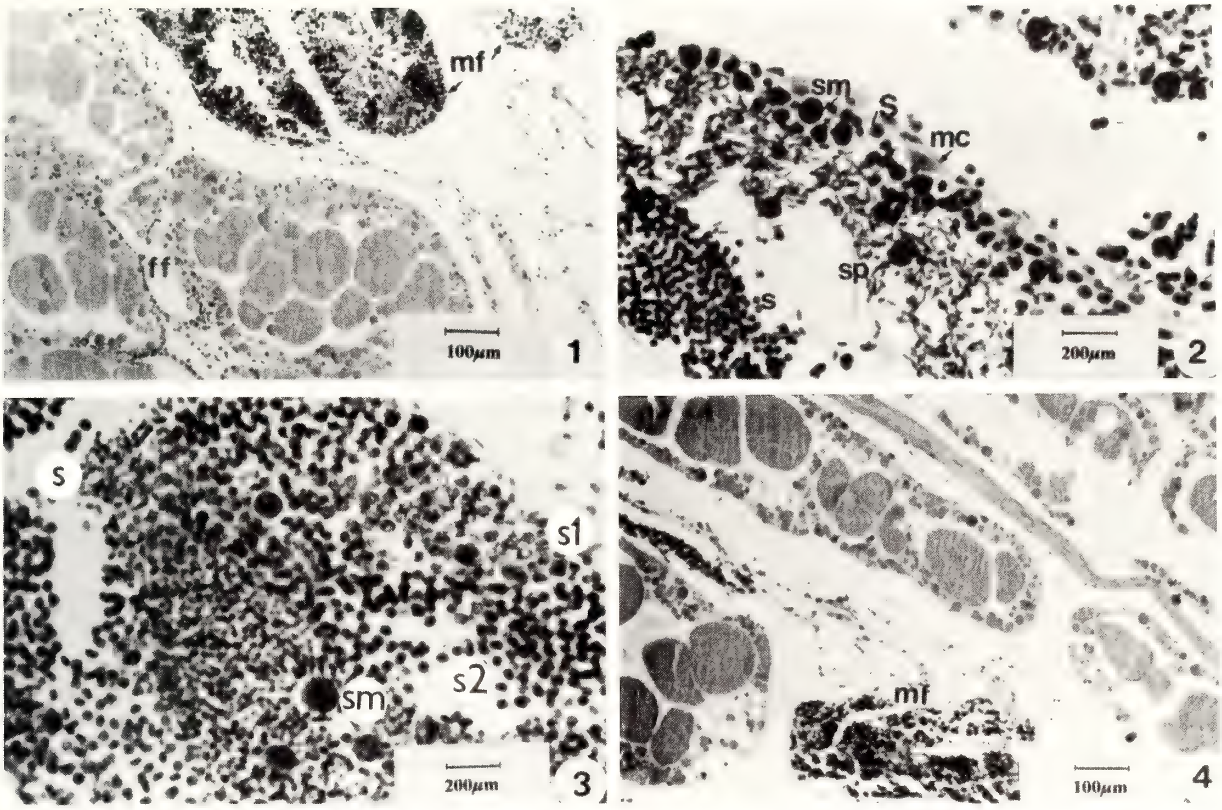
In general, gametogenesis in *Diplodon rotundus gratus* is similar to that described by Peredo and Parada (1984, 1986) for *D. chilensis chilensis*. *D. rotundus gratus* is a hermaphrodite, with the gonad located in the visceral mass between the loops of the alimentary canal (Hebling and Penteado, 1974). The female portion occupies the lower two-thirds of the visceral mass, extending from the region of the digestive diverticula in front of the stomach to the posterior end of the foot. The male portion occupies the upper third of the visceral mass, extending from the ventral region backward to that below the renopericardial complex. Both portions consist of irregular tubes or branches. These sac-like follicles or acini differ in size and shape and are always delimited and enveloped by a follicular membrane.

Inside the follicles are the male and female reproductive cells (Fig. 1), present in various phases of development and recognizable by their shape, size, and other characteristics. The developmental stages observed in *D. rotundus gratus* were similar to those described by Jones *et al.* (1986), with some modifications. Follicular Stage I is the beginning of gametogenesis and the phase of follicle formation. Components include: (1) precursor cells (mother-cells; Fig. 2), large and irregular cells with weak chromatin in parallel to the follicular wall, which give rise to spermatogonia or oogonia after the first division; (2) spermmorulae in males (Figs. 2-3), dark multinucleate spheres usually at the periphery of the follicle, defined in *D. chilensis chilensis* by Peredo and Parada (1984); and (3) nutritive cells in females (Fig. 5). Follicular Stage II is the active phase of gamete production and maturation (Fig. 3). Follicular Stage III is the phase of gamete elimination (spawning) to the gonoducts, defined here in two subdivisions: IIIa, partial elimination, and IIIb, extensive elimination and end of gametogenesis. Follicular Stage IV is the period of recovery following gametogenesis. In the male (Fig. 4), follicles are quite reduced in number and size or almost totally absent; a few cells occasionally remain inside the follicle (Fig. 4). In the female, Stage IV follicles exist in three conditions: (1) small gonad with fewer follicles of reduced size with abundant interfollicular tissue [remaining oocytes assumed to be eliminated or to suffer lysis (Fig. 6)]; (2) follicles initiating a new cycle where nutritive cells are abundant; some oocytes still present and some follicles undergoing lysis; (3) all follicles in the process of lysis (Fig. 7).

MARSUPIUM AND GLOCHIDIA

After fertilization, zygotes migrate to the inner demibranchs (marsupia) where they develop and transform into glochidia. Glochidia, in turn, require a host to complete their life cycle. Development is completed through parasitism of fish that occur in the region. Fish captured in the Pardo River included species of *Astyanax, Curimata, Holochestes, Piabina, Steindachnerina, Pimelodella, Lebistes, Geophagos, Leporinus*, and *Eingenmannia*, all of which could have been infested. The most intensive infestations were observed in the species *Steindachnerina insculpita* (Fernández-Yépez, 1948), *Holochestes heterodon* (Eigenmann, 1915), and *Pimelodella* sp. The highest infestation of these fishes occurred in April.

When living bivalves were examined in the laboratory, the demibranchs were observed to differ in color. Some were whitish, some dark brown, and others were lighter brown with small white spots. Microscopic examination of white demibranchs revealed only developing embryos still within the vitelline membrane. Dark brown demibranchs were filled with fully differentiated glochidia, with larval shells, adductor muscles, and glochidial hooks. Lighter brown demibranchs contained a mixture of devel-



Figs. 1-4. *Diplodon rotundus gratus*, histological sections. 1. General aspect of gonad showing male and female follicles. 2. Spermatogenesis. 3. Male follicle in Follicle Stage II. 4. Male follicle in Follicle Stage IV. (ff, female follicle; mc, mother-cell; mf, male follicle; s, spermatid; S, spermatogonia; s1, first-order spermatocyte; s2, second-order spermatocyte; sm, sperm-morulae; sp, spermatozoon).

oping embryos as well as fully differentiated glochidia.

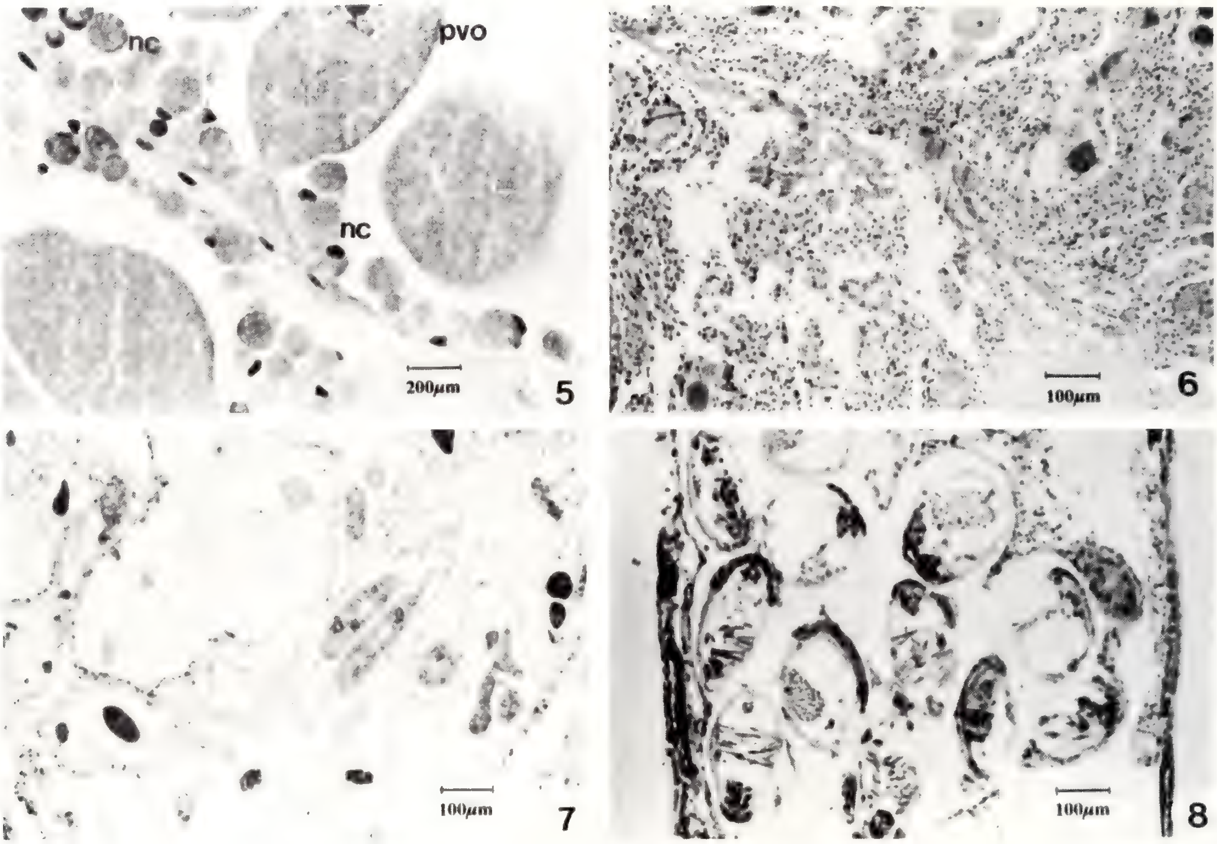
Examination of histological sections allowed observation of marsupia with empty demibranchs, with embryos, and with fully developed glochidia. All phases were histologically well-defined but when the marsupium was empty, two conditions were observed: a preincubation phase characterized by thickening of the inner demibranch wall, and a second, post-incubation phase in which the marsupium was small, with less-thickened walls, and occasionally presenting bubble-like empty spaces.

Three developmental stages of the marsupium were defined for analytical purposes. Marsupial Stage I included empty marsupia, *i.e.* the absence of embryos or glochidia, including those ready to receive fertilized oocytes (preincubation phase) or those that had already eliminated glochidia (post-incubation phase). Marsupial Stage II included incubating marsupia, containing embryos in various phases of development still surrounded by vitelline membranes even when almost fully mature. Marsupial Stage III comprised marsupia containing glochidia free from the vitelline membrane, recognized by the presence of adductor muscles, glochidial hooks, and larval shells (Fig. 8).

REPRODUCTIVE CYCLE

Monthly analysis of histological sections revealed two periods in the reproductive cycle of *Diplodon rotundus gratus*, one from February to July, and the other from August to January. The first period was characterized by predominance of both male and female follicles simultaneously manifesting active oogenesis and spermatogenesis, with large numbers of sperm-morulae and nutritive cells (Fig. 5) in their respective follicles at the beginning of this period. Beginning in June, the number of functional hermaphrodites decreased gradually, with an increase in the number of functionally female individuals (Fig. 9).

According to follicular development in the histological sections, marsupia were in most cases initially empty (Marsupial Stage I), with gradual growth in terms of the presence of embryos and glochidia, peaking in Marsupial Stages II and III during the months of May and August (Fig. 10). When marsupial smears were analyzed, synchrony was noted between the stages of incubation observed in histological sections and those in the smears (Fig. 11). In April there was clear evidence of extensive elimination of both male and female gametes (Follicular Stage IIIb) (Figs. 12-14). After intense and rapid production



Figs. 5-8. *Diplodon rotundus gratus*, histological sections. 5. Female follicles in Follicular Stage I. 6. Female gonad in Follicular Stage IV with interfollicular tissue. 7. Female follicles in Follicular Stage IV showing lysis of oocytes. 8. Marsupium with glochidia. (nc, nutritive cell; pvo, previtellogenic oocyte).

of gametes in February and March, with abundant sperm-morulae and nutritive cells, considerable reduction in the latter two was observed beginning in April, with a consequent reduction in gametogenesis.

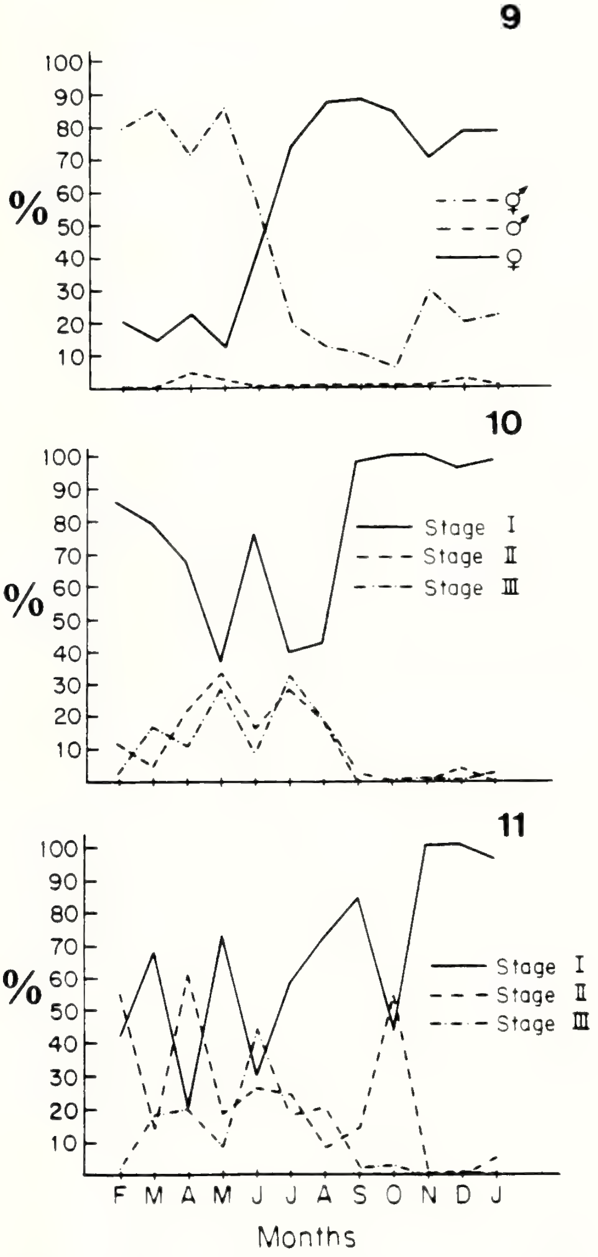
In the second period of reproduction (August to January), more and more specimens were functionally female as determined by histological examination, with the highest percentages occurring in August, September, and October (Fig. 9). In contrast, beginning in November, there was an increase in the percentage of functional hermaphrodites accompanied by a concomitant reduction of functional females. With respect to gonadal development, beginning in August, both male and female follicles of virtually all specimens were in Follicular Stage IV.

Marsupial differentiation observed in the histological sections showed that many embryos and glochidia (Marsupial Stages II and III, respectively) were present in large numbers in August and that Marsupial Stage I (post-incubation phase) began to predominate in September, with a clear decrease in inner demibranch size. During this period there was also synchrony between the stages of gonadal development and marsupial development, except in October 1991 when the percentage of individuals with marsupia containing immature glochidia reached 54% (Figs. 10-11). The number of nutritive cells present in female follicles decreased gradually until August and began to gradually increase again beginning in September, coinciding with the spring and summer seasons of more intense rainfall.

Sperm-morulae in male follicles (Figs. 2-3) were abundant in February and March, markedly decreased from April to October, and increased beginning in November, leading to maximum values in February and March (Table 1). Only small percentages of the collected specimens had non-reproductive follicles: 2% in June, 7% in July, and 0.2% in September.

DISCUSSION

According to the classification of Coe (1943), *Diplodon rotundus gratus* is a functional hermaphrodite. In the population studied here, hermaphroditism was observed

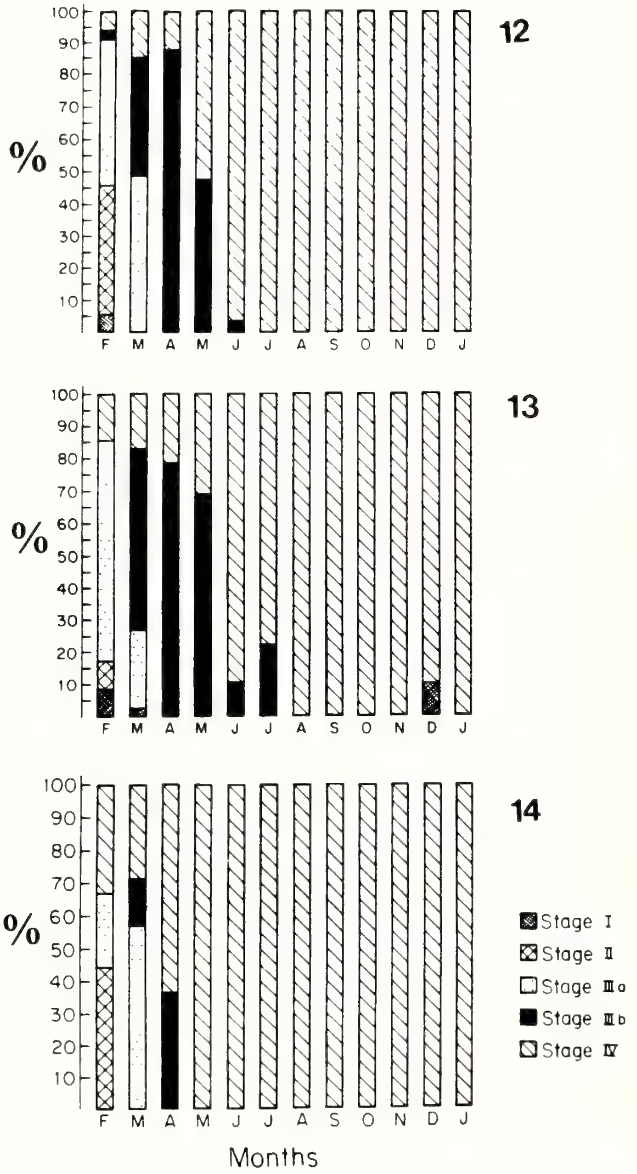


Figs. 9-11. *Diplodon rotundus gratus*, annual percentages of individuals collected over one year (February 1987 to January 1988, Figs. 9-10, and February 1991 to January 1992, Fig. 11). 9. Percentages of hermaphroditic, male, and female individuals. 10. Percentages of individuals in Marsupial Stages I-III as observed in histological sections. 11. Percentages of individuals in Marsupial Stages I-III as observed in marsupial smears.

in practically all months of the year, with higher percentages at the end of summer and in the fall. In winter and spring the population consisted of individuals with mostly female follicles, followed by a new gradual increase in hermaphrodites beginning in November.

In *Diplodon rotundus gratus*, as also in *Argopecten irradians* (Lamarck, 1819) (*fide* Coe, 1943), spermatic tissue occupies the dorsal portion of the gonad with ovarian tissue in the ventral portion. The two portions are not morphologically demarcated. Follicles simultaneously presenting male and female elements are rare and there is no difference in color between the two follicle types.

Two types of spermatogenesis occur in *Diplodon rotundus gratus*: in the first, the mother-cell undergoes nor-



Figs. 12-14. *Diplodon rotundus gratus*, annual percentages of individuals collected over one year (February 1987 to January 1988) in Follicular Stages I-IV as observed in histological sections. 12. Oogenesis in hermaphroditic specimens. 13. Spermatogenesis in hermaphroditic specimens. 14. Oogenesis in functionally female specimens.

Table 1. *Diplodon rotundus gratus,* percentages of hermaphrodites plus functional males (H + M) among total individuals (N) collected with sperm-morulae present or absent, over a period of one year (February 1987 to January 1988).

Month	N	H + M	% present	% absent
February	44	35	75	5
March	48	40	48	38
April	46	34	4	72
May	49	43	6	82
June	50	27	2	54
July	46	8	4	15
August	49	5	4	8
September	50	5	6	4
October	38	6	3	13
November	50	15	26	2
December	50	11	8	14
January	50	11	16	6

mal cell division giving rise to first- and second-order spermatocytes, spermatids, and spermatozoa. In the second type, the mother-cell divides mitotically and the resulting cells remain joined with an intrafollicular arrangement resembling that of a morula stage (from which the term sperm-morulae was derived); the sperm-morulae, in turn, undergo meiosis, giving rise to spermatocytes, spermatids, and spermatozoa. This second type results in larger numbers of spermatozoa, perhaps as an adaptation in response to the short reproductive period of these bivalves.

The developing oocytes are connected to the follicular wall by a peduncle and are nourished by nutritive cells until they mature. Mature oocytes that are not released and that remain in the follicles after the elimination phase are reabsorbed by lysis from August to February. Jones *et al.* (1986) described a similar case in *Cucumerunio novahollandiae* (Gray, 1834).

Analysis of the marsupia of *Diplodon rotundus gratus* revealed the absence of an incubation period in spring or summer, confirmed by the almost total absence of embryos and glochidia. During this period, only female follicles exist due to total regression of male follicles. However, the marsupial smears of animals collected in 1991 showed the existence of an incubation peak in October, with about 54% of the individuals presenting embryos in the marsupium, in contrast to the marsupia observed in histological sections.

Temperature could be a factor determining spawning activity. Several investigators have demonstrated that the spawning activity of marine bivalves is directly related to temperature (Chipperfield, 1953; Allen, 1962; Wilson and Hodgkin, 1967; Lunetta, 1969). In *Diplodon rotundus gratus*, the spawning period begins in March when water temperature decreases. At the end of July, when the lowest temperatures occur, spawning virtually ceases and the animals enter a process of recovery and follicular rearrangement that extends throughout August, September, October, and the beginning of November.

Gametogenesis begins at the end of spring and continues during the summer, coinciding with the period of heavy rainfall when the water volume of the river increases considerably and temperatures reach high levels. Jones *et al.* (1986) described a similar cycle in Australian limnic bivalves.

The duration of the period of incubation in *Diplodon rotundus gratus* is similar to that in several unionaceans from the temperate zone. Organogenesis in *D. rotundus gratus* lasted about 30 days. Yokley (1972), Yager and Neves (1986), and Bruenderman and Neves (1993) also observed short periods of incubation in limnic bivalves in the Northern Hemisphere.

Only one reproductive peak was observed in *Diplodon rotundus gratus*, in contrast to *D. chilensis chilensis*, in which reproduction is a continuous process (Peredo and Parada, 1986). In *D. rotundus gratus*, there is synchrony in reproduction and in male and female gamete maturation, as also recorded by Jones *et al.* (1986) and Peredo and Parada (1984, 1986).

Fish captured by this study and found to be infested with glochidia coincidentally occurred at the end of summer and fall, demonstrating that the species of Hyriidae occurring in the Pardo River present cycles similar to that in *Diplodon rotundus gratus*. Unfortunately it was not possible to identify the glochidia parasitizing these fish, but more detailed studies of the life cycle of these bivalves are currently being planned.

ACKNOWLEDGMENTS

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Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system

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Abstract: An indoor recirculating aquaculture system was constructed to provide suitable conditions to culture juvenile freshwater mussels. In the first of three growth trials, *Villosa iris* (I. Lea, 1829) juveniles were cultured for 22 wk, and grew from an initial mean length of 0.4 mm to 2.7 mm. Survival was 26.8% overall. In the second trial, growth and survival were compared between juveniles of *V. iris* held in sediment and without sediment. The initial mean length of both groups was 2.7 mm, and this experiment ran for 17 wk. The juvenile mussels in sediment grew to a mean length of 5.7 mm with 85% survival, significantly greater ($p < 0.01$) than juveniles held without sediment (4.5 mm, 74% survival). In the third trial, two cohorts of juvenile *Lampsilis fasciola* Rafinesque, 1815, increased in length from 1.1 mm and 1.4 mm to 3.3 mm and 4.1 mm, respectively, with comparable survival (78.7% versus 64.5%). Results of these trials demonstrate that juvenile mussels can be reared successfully within recirculating systems. One of the factors deemed important in successful culture is continuous feeding of an appropriate food source. In this study, a unialgal culture of *Neochloris oleabundans* Chantanachat and Bold, 1962, was used throughout. Regular cleaning of the system and water replacement also was important. Finally, the culture of juveniles in sediment appears to be an important factor in ensuring good growth and survival. This phenomenon could be related to pedal feeding behavior, proper orientation of the mussels for filtering efficiency, or stability from physical disturbance.

Key words: Unionidae, freshwater mussels, *Villosa, Lampsilis*, algae, recirculating aquaculture

Major declines in freshwater mussel populations (Unionidae) were recorded by the early twentieth century (Smith, 1899; Coker *et al.*, 1921) and were attributed primarily to overfishing for a burgeoning pearl button industry (Coker, 1919). More recently, declines in unionid numbers have been noted and ascribed to pollution, habitat destruction or alteration, competition from non-indigenous bivalve species [*e. g.* Asian clam, *Corbicula fluminea* (Müller, 1774), and the zebra mussel, *Dreissena polymorpha* (Pallas, 1771)], as well as to harvest for shell to produce nuclei for pearl culture. The realization of this dramatic decline by resource managers and scientists within the last two decades has prompted an increase in much needed studies on basic life history and ecology of numerous unionid species. Such studies continue as more is learned of environmental and host-fish requirements for survival and reproduction in this group of bivalves (Isom and Hudson, 1982; Zale and Neves, 1982; Neves and Widlak, 1987; Dimock and Wright, 1993).

Historically, reports on the aquaculture of juvenile unionids have conveyed little detailed empirical information. Many of the data are anecdotal, with little or no monitoring of the progress of the juveniles throughout the culture period (Lefevre and Curtis, 1912; Coker *et al.*, 1921; Howard, 1922). Culture attempts on unionids using artificial media have resulted in some success (Isom and Hudson, 1982; Keller and Zam, 1990). However, the application of these results to the large-scale and long-term propagation of unionids remains speculative and untested. Artificial culture of endangered and threatened mussel species has been strongly recommended in recovery plans as a strategy to enhance declining population numbers, as well as for the reintroduction of species to sites within their historic ranges.

With these propagation needs in mind, we initiated a project focusing on factors influencing the growth and survival of juvenile unionids in a captive environment. To this end, a recirculating system was constructed to rear juvenile mussels under controlled conditions of food rations, water temperature and flow, and to monitor growth and survival. This report describes feeding and maintenance protocols for the recirculating culture system and presents results of growth and survival trials with juvenile mussels.

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MATERIALS AND METHODS

The recirculating culture system was located within a greenhouse facility at the Virginia Tech Aquaculture Center, Blacksburg, Virginia. The greenhouse has the capability of maintaining fairly constant temperature either by the use of an evaporative cooling system or propane heaters. However, water temperatures throughout the study, while differing from ambient temperatures especially in winter months, exhibited some seasonal fluctuation.

The recirculating system used for culturing juvenile mussels consisted of a rectangular reservoir tank (225 l) from which water was pumped by a 1/25 hp magnetic drive pump through half-inch PVC line, to one end of an elongated raceway-type tank, which served as the primary culturing chamber. The raceways were 3 m in length and 66 cm in width. Water flow into the raceway was regulated and measured using an in-line flow meter. Water was introduced into the raceway by ten parallel jets across the width of the chamber, which provided adequate uniformity in flow within the system. The water in the raceway was gravity-fed back to the reservoir via a standpipe. Water capacity in the raceway could be varied by adjusting the height of the standpipe. Aeration in the system was provided by air pumped into the reservoir and supplemented by the pumping action. Throughout the growth trials, the water depth in the raceway was 20 cm, giving a volume of 170 l.

Municipal water was conditioned (dechlorinated) by aeration for a period greater than 24 hr prior to use in the culturing system. Because the municipal water was soft (< 55 mg/l $CaCO_3$), hardness of the water was increased by the addition of well-water (hardness of 450 mg/l $CaCO_3$) to maintain an overall hardness in the system at ca. 200 mg/l $CaCO_3$.

Throughout the growth trials, juvenile mussels were fed the unicellular green alga *Neochloris oleoabundans* Chantanachat and Bold, 1962, which has been shown to support good growth in juvenile mussels (Gatenby, 1994). Mussels were fed algae at a density of 10,000 cells/ml either once or twice daily. Ultraviolet-sterilized and dechlorinated water was used for algal cultures and the recirculating system. Living algal cultures were maintained semi-continuously in 250 l Kalwall clear plastic tubes (Kalwalls Aquacenter, Leland, Mississippi). The unialgal cultures were not axenic. Enrichment of the algal culture was achieved by the addition of appropriate quantities of nutrient media (Ukeles, 1971). We attempted to harvest algae at the late exponential growth phase, although this might not have been achieved for all feeding events.

Mussel Species

The growth trials described herein were conducted on common species of mussels to determine the potential success of such a system on endangered species. One surrogate for these rare species is the rainbow mussel, *Villosa iris* (I. Lea, 1829), common to streams and rivers of the upper Tennessee River system. This species was selected because of its relative abundance, and the success with which large quantities of juveniles can be acquired from host fishes. Also, the habitat requirements of *V. iris* (riffles and shoals) are similar to those of many endangered species. In addition, two cohorts of the wavy-rayed lampmussel, *Lampsilis fasciola* Rafinesque, 1820, were utilized in growth trials. This species also is common to the upper Tennessee River system. Fish infestation and collection of juveniles were accomplished by standard techniques (Zale and Neves, 1982). Rock bass (*Ambloplites rupestris* Rafinesque, 1817) were infested with glochidia of *V. iris*, and largemouth bass (*Micropterus salmoides* Lacépède, 1802), were used as the host fish for *L. fasciola*.

Growth Trial 1

Young rainbow mussels with a mean shell length of 0.4 mm were placed in three petri dishes (18 cm x 1 cm) with 600-700 mussels per dish. Fine sediment (< 120 µm), collected from the Clinch River at Carbo, Virginia, and aerated during storage, was placed in each dish to a depth of 1-1.5 mm. The introduction of feed to the mussels in this first trial was not regimented, as the primary purpose was to fine-tune the system, in terms of adjusting flow and ensuring good circulation. The flow rate of water through the raceway was 6 l/min. Growth (shell length) was assessed at 2, 4, 8, 16, and 22 wk of the trial from a sample of juveniles seived from the sediment. Fresh sediment was placed in the culture dishes after each sampling event. Water was changed in the system every 10-12 d (with one exception). This pilot study was initiated in August and terminated in December 1996.

Growth Trial 2

A second trial followed the first, using those mussels surviving the first trial supplemented with mussels of the same age from another study. Given the relatively small number of animals held in the system, it was anticipated that water quality within the system would not be seriously compromised. However, water was changed on a weekly basis and water quality variables were more closely monitored in this study. On a weekly basis, temperature, pH, hardness, un-ionized ammonia, and dissolved oxygen were measured. Also, older juveniles require a greater rate of water change (flow) than younger individuals (Jiang Li-Fan, Freshwater Fisheries Research Center, Wuxi, P. R. China, pers. comm.), therefore, flow rate across the dishes was increased to 9 l/min.

Two culture techniques were evaluated within the system during this trial. The first culture method employed a sediment substratum (< 350 µm). Three replicate petri dishes contained sediment to a depth of 0.5-0.7 cm. The growth of juveniles in these dishes was compared to that of juveniles in dishes (N = 3) without substratum. The rationale for this comparison was to determine whether sediment was a necessary component in the survival and growth of juvenile mussels at this particular size (age) in their development. If not, then sediments could be discarded from the culture protocols, thus making maintenance and cleaning of the culture system much easier. Upon initiation of the second growth trial, 100 juveniles were placed within each petri dish. Growth (length of 30 juveniles per dish) and survival (total living count per dish) were monitored every 2 wk for the duration of the study (17 wk).

Growth Trial 3

Two cohorts of juvenile *Lampsilis fasciola* were placed in the recirculating system to evaluate their growth and survival with the rearing protocols previously described. At the start of this trial, cohort 1 had a mean size of 1.1 mm. Growth and survival were estimated four times over the subsequent 12 wk. Cohort 2 had a mean size of 1.4 mm upon initiation of this trial, and these mussels were sampled only twice during the 12 wk period.

RESULTS

Growth Trial 1

Juvenile mussels in the three dishes, at an initial mean shell length of 0.4 mm, exhibited a steady rate of growth for the 22 wk period, achieving a mean length of 2.7 mm (Fig. 1). Survival after the first two sampling periods (4 wk) was high (approx. 95%) in all dishes. However, between the second and third sampling interval (8 wk), high mortality (60-65%) was observed in all dishes. Thereafter, mortality was low, concluding with an overall survival of 26.8%. Water temperatures in the dishes from October to December ranged from 24.5-15.4°C, with a steady decline as winter progressed.

Growth Trial 2

The mean lengths of juvenile mussels at the start of growth trial were identical (mean = 2.7) for the sediment and no-sediment treatments (Fig. 2). Growth for the first two sampling periods (4 wk) was slow for both treatments; however, after 6 wk, a discernible difference was apparent between the two treatments. The mean size of juveniles in the sediment treatment was 3.5 mm, whereas that of juveniles in the no-sediment treatment was 3.1 mm. This difference was further exaggerated after 9 wk, when the mussels in the sediment and no-sediment treatments had mean sizes

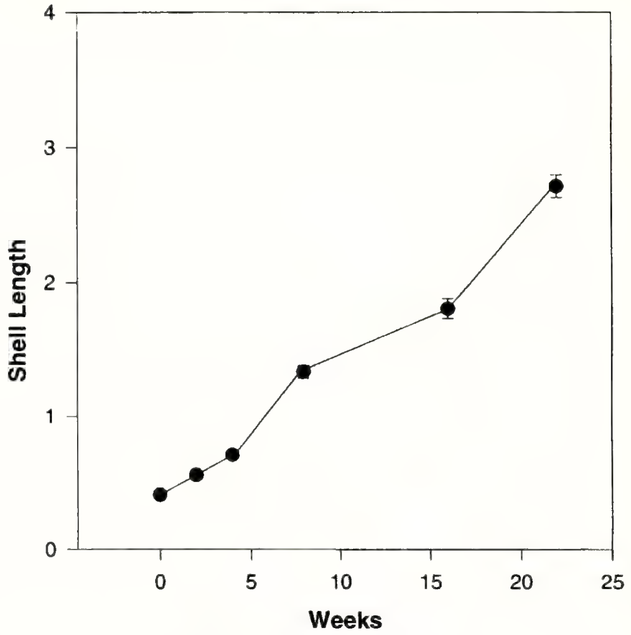

Fig. 1. Mean shell length (mm ± 2 SE) of juvenile rainbow mussels, *Villosa iris*, over 22 wk (Trial 1).

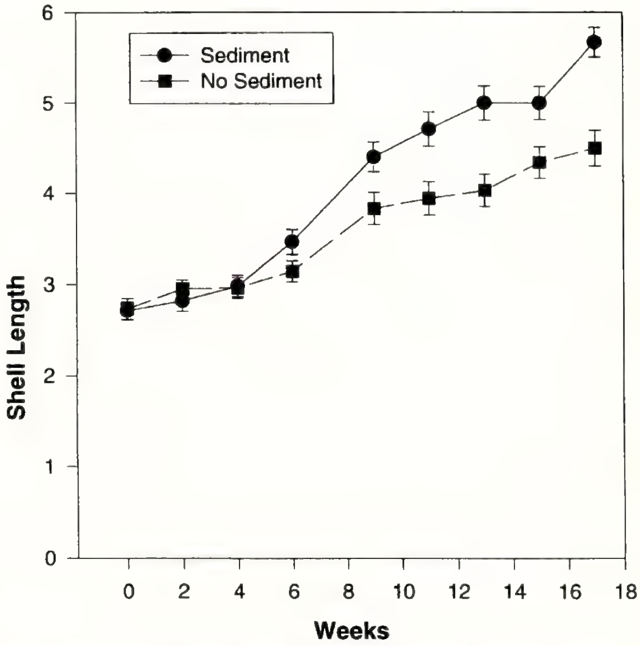

Fig. 2. Mean shell length (mm ± 2 SE) of juvenile rainbow mussels, *Villosa iris*, in sediment and no-sediment treatments over 17 wk (Trial 2).

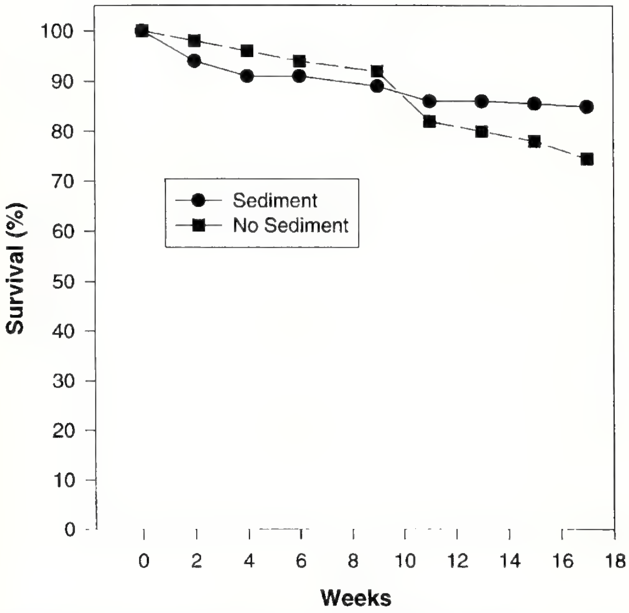

Fig. 3. Mean survival of juvenile rainbow mussels, *Villosa iris*, in sediment and no-sediment treatments over 17 wk (Trial 2).

of 4.4 mm and 3.8 mm, respectively. Upon completion of the study at 17 wk, the mussels held in sediment were 5.7 mm; no-sediment mussels had attained a mean size of only 4.5 mm. Repeated measures Analysis of Variance on these data, after termination of the study, indicated that the mussels held in sediment were significantly larger than those held without sediment ($p < 0.01$). Overall, survival also differed between the two treatments (Fig. 3). Those juveniles held in sediment had an overall survival of 85%, whereas the no-sediment treatment mussels exhibited significantly lower survival (74%). It was noted throughout this study that many juveniles held in sediment attached to the container surface and each other by byssal threads. This trait made separation for measuring and enumeration difficult. Conversely, those mussels cultured in dishes without sediment produced no byssal threads at any period in the study.

Growth Trial 3

Cohort 1 had grown to a mean size of 2.3 mm after 4 wk and 3.3 mm after 12 wk, when the trial was terminated (Fig. 4). Cohort 2 had grown to 3.9 mm after 10 wk and to 4.1 mm by 12 wk. Survival of cohort 1 and cohort 2 after 12 wk was 78.7% and 64.5%, respectively.

Water Quality

Water temperatures throughout the study fluctuated with air temperatures and ranged between 15.4°C and 26.4°C. The overall mean water temperature in the culture system was 20.6°C. Dissolved oxygen (mg/l) and pH values remained relatively stable throughout the study; both had mean values of 8.3. Un-ionized ammonia showed some variability, ranging from 0 to 0.053 mg/l, with a mean value of 0.013 mg/l. Water hardness had a mean value of 213 mg/l $CaCO_3$, close to the targeted value of 200 mg/l $CaCO_3$. However, there was considerable variation in hardness values between samplings (range 138-277 mg/l $CaCO_3$).

DISCUSSION

The growth of *Villosa iris* in Trial 1 was notable; 2.7 mm after 22 wk. This rate of growth exceeds that of 2.9 mm after 39 wk (Gatenby *et al.*, 1996), and 1.8 mm after 20 wk under different culture conditions for the same species (Gatenby *et al.*, 1997). The rainbow mussel is a small, slow-growing species in the Tennessee River system. When compared to other estimates of growth rates observed in the wild, the growth rate and sizes recorded in this study were considerably greater than those previously reported. Neves and Widlak (1987) estimated the mean size of juvenile freshwater mussels from a variety of species (including *V. iris*) as 2.7 mm after 1 yr (52 wk). Survival of our juvenile mussels also was comparable to those reported in previous studies. Gatenby *et al.* (1996) reported 8% survival of *V. iris* after 165 days, and 30-38% survival after 140 days under their best culture conditions (Gatenby *et al.*, 1997). After 154 days, the mean survival in growth trial 1

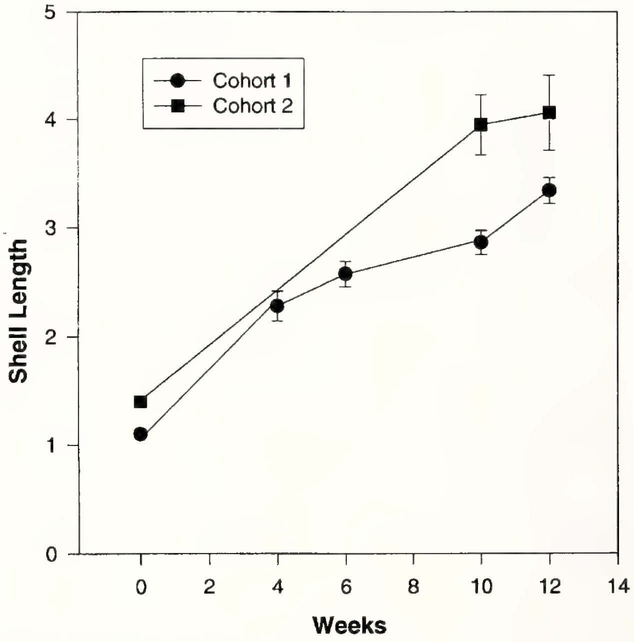

Fig. 4. Mean shell length (mm ± 2 SE) of two cohorts of the wavy-rayed lampmussel, *Lampsilis fasciola*, over 12 wk (Trial 3).

was 26.8%, which included the large single mortality event that occurred between the second and third sampling periods. The exact cause of this high mortality event is unknown; however, it is our opinion that the cause was poor water quality due to an oversight. Because the scheduled water change did not occur between the second and third sampling periods, the sediments were fouled with filamentous algae, previously shown to result in high juvenile mussel mortality (Yang, 1996). The water quality condition in the interstitial sediments, while unknown, was also assumed to be less than optimum. Juveniles of even eurytopic species such as *Utterbackia imbecillis* (Say, 1829) and *Pyganodon cataracta* (Say, 1817) have been shown to be very sensitive to conditions of hypoxia and low pH, as well as elevated temperatures (Dimock and Wright, 1993).

The performance of juvenile mussels in growth Trial 2 also proved informative. The significantly greater growth of mussels held in sediment as compared with those with no sediment was somewhat surprising. We compared growth and survival of juvenile rainbow mussels cultured with and without sediment in this trial. If comparable or better growth was achieved without the sediment, then the use of sediment could be dispensed with, which would make the maintenance and cleaning of the culture systems easier. However, this was not the case. Those animals held in sediment had greater growth and survival than those juveniles with no substratum at all. The reason for this phenomenon is unclear; however, we offer some possible reasons for the differences observed. Juvenile mussels could obtain nutrition from the sediment, to include organic matter or micro-organisms. The substratum also allows juvenile mussels to orient themselves properly for feeding. By doing so, they can siphon from higher in the water column and avoid the slower water in the boundary layer near the sediment-water interface (Vogel, 1981; Mann and Lazier, 1991). The mussels in the sediment were clearly seen to orient themselves in this manner, whereas the mussels without sediment lay on one valve with no vertical orientation.

The sediment offers a certain amount of stability, which would buffer juvenile mussels from disturbance by turbulent water flow or vibrations originating outside the culture vessels. Those juveniles held without sediment were subjected to greater disturbance, as witnessed by the buffeting (from both flow and vibrations) that they received in the dishes. When disturbed in such a manner, the mussels had a tendency to cease feeding and close up. This cessation of feeding could result in their slower growth and reduced survival. The response of the wavy-rayed lampmussel cohorts in growth trial 3 seems to support this disturbance hypothesis (Fig. 4). Both cohorts appeared to have similar growth rates until the first sampling of cohort 1. Thereafter, the growth of this cohort appeared to decrease dramatically with further samplings. The first sampling of cohort 2 did not occur until week 10, after which the growth rate decreased appreciably. Sampling involved removal of the mussels from water and much handling for periods in excess of 20 min. Stress associated with the handling of juvenile mussels could have contributed to a reduction in growth. We speculate that disturbance associated with the sampling of these juveniles (measuring and counting) had a detrimental effect on the mussels. The juvenile mussels held without sediment would undoubtedly be exposed to greater disturbance, and this could be reflected in reduced growth rate, as we observed. Vanderploeg *et al.* (1995) determined that filtration rate in *Lampsilis radiata siliquoidea* (Barnes, 1823) was comparable between mussels in sediment and those lying on their sides. However, they used adult mussels and warned that such a phenomenon might not apply to all unionids. It has long been recognized that filtration rate in bivalves is adversely affected by mechanical or physical disturbance (Jorgensen,1960; Mohlenberg and Riisgard, 1979).

The degree of stress that young mussels experienced in our study can also be evidenced by the fact that the mussels held in sediment produced copious amounts of byssal threads to secure themselves to the bottom of the culture dish. Conversely, the mussels held without sediment produced no byssal threads. After the study was completed, the mussels held without sediment were placed in sediment, and within 1 wk they produced byssal threads. We hypothesize that the sediment provided appropriate habitat for the mussels, which consequently produced byssal threads to secure themselves within the substratum. Those held without sediment were in unstable habitat and did not produce byssal threads. A conclusion of our study is that young freshwater mussels, without suitable substratum, do not exhibit high growth and survival. This is supported by evidence from marine mussels, *Mytilus* spp., where production of byssal threads is mediated by the favorability of the habitat encountered (Morton, 1992), and is a result of net energy surplus (Hawkins and Bayne, 1992).

The water quality parameters recorded throughout the study were not detrimental to juvenile mussels (Table 1). Temperature fluctuations, despite their range, were gradual and well within the range that juvenile mussels experience in the rivers and streams of southwestern Virginia. Hardness levels also fluctuated considerably, yet the values were such that the water was considered moderately hard (Landau, 1992). Dissolved oxygen and pH values remained relatively stable and were not deemed stressful to the juveniles mussels. Un-ionized ammonia levels were considerably lower than levels reported to cause mortality in the marine northern quahog, *Mercenaria mercenaria* (Linné, 1758) (see Stevens, 1982).

Despite the differences observed in growth Trial 2, the growth and survival in both treatments with *Villosa iris*

Table 1. Summary of water chemistry during juvenile mussel culture experiments.

Date	Temperature (°C)	Dissolved Oxygen (mg/l)	pH	Un-ionized Ammonia (mg/l)	Hardness (mg/l $CaCO_3$)
10/25/96	21.0	7.8	8.5	0.013	255
10/31/96	24.6	7.4	8.4	0.008	138
11/5/96	21.7	7.9	8.4	–	160
11/8/96	22.5	7.9	8.2	–	–
11/14/96	15.4	9.1	8.2	–	250
11/18/96	20.0	7.6	8.6	–	–
11/22/96	20.8	8.6	8.7	0.037	235
11/30/96	17.4	9.3	8.6	0.022	235
12/14/96	19.7	9.3	8.5	0.007	205
12/22/96	15.7	9.6	8.8	0.000	193
12/28/96	23.1	8.6	8.6	0.043	243
1/4/97	26.4	7.5	8.9	0.053	249
1/11/97	18.9	9.4	8.6	0.040	206
1/18/97	15.9	10.9	8.4	0.000	220
1/24/97	17.9	8.9	8.1	0.002	201
2/2/97	23.0	8.4	8.4	0.001	190
2/8/97	19.5	8.7	8.0	0.001	201
2/16/97	17.2	9.9	8.5	0.008	277
2/21/97	24.6	7.5	8.4	0.003	264
3/7/97	19.5	–	–	–	200
3/16/97	17.9	8.8	8.3	0.025	190
3/21/97	24.0	5.8	8.3	0.001	167
3/31/97	18.3	9.0	7.3	0.000	144
4/4/97	22.5	6.1	8.0	0.001	230
4/14/97	20.9	6.5	7.8	0.001	261
4/22/97	20.9	7.9	7.5	0.000	215
Mean ± SD	20.4 ± 2.9	8.3 ± 1.2	8.3 ± 0.4	0.013 ± 0.0016	213 ± 37.9

were excellent, and matched or exceeded the rates observed in previous culture attempts (Yang, 1996; Gatenby *et al.*, 1997). The commercial culture of marine bivalves is considered successful if a survival rate of 1-5% is realized from egg to planting size (M. Castagna, Virginia Institute of Marine Science, Eastern Shore Laboratory, pers. comm.; M. Pierson, Cherrystone Aquafarms, Cheriton, Virginia, pers. comm.). Our culture attempts compare favorably with these estimates. However, it must be noted that brooding organisms (*e. g.* freshwater mussels) tend to exhibit higher survival than non-brooding planktotrophic species (*e. g.* many marine bivalves). The growth and survival observed with the two cohorts of *Lampsilis fasciola* was encouraging, and provided evidence that the system and techniques employed in these studies can be applied successfully to other species of freshwater mussels. No single factor can be identified as causative in the success of our culture attempts. It is our opinion that the combination of the system (providing unidirectional consistent water flow with adequate dissolved oxygen levels) and the husbandry techniques (daily feeding and regular cleanings) were extremely important for the successful culture of the juvenile unionids. It has long been appreciated that successful culture of marine bivalves can only be achieved by vigilant monitoring, cleaning, and constant feeding through the larval and juvenile phases (Castagna and Kraeuter, 1981). The ability to consistently rear large numbers of freshwater mussels, from the juvenile stage to a size suitable for relocation to natural environments, is a much-needed step in the conservation of rare unionids or the production of commercially valuable species. Results of our study demonstrate that this goal is achievable with further research into the factors influencing the growth and survival of freshwater mussels within indoor culture systems.

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Feeding interactions between native freshwater mussels (Bivalvia: Unionidae) and zebra mussels *(Dreissena polymorpha)* in the Ohio River

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Abstract: The effects of zebra mussel infestation on the feeding of native unionids in the Ohio River were evaluated through gut contents and available food in the water column. In 1996, heavily infested *Amblema plicata* (Say, 1817) and *Quadrula pustulosa* (I. Lea, 1831) had significantly less ($p < 0.01$) organic matter in their guts (1.4 and 0.6 mg ash-free dry weight [AFDW], respectively) than lightly infested specimens (4.6 and 1.8 mg AFDW, respectively), and heavily infested *Q. pustulosa* had a significantly lower ($p < 0.05$) mean algal cell number (1.8×10^4) in the guts than lightly infested specimens (3.9×10^5). However, mean algal cell numbers in the guts of heavily infested and lightly infested *A. plicata* (5.7×10^5 versus 9.1×10^5, respectively) were not significantly different ($p = 0.17$). In 1997, significant reductions ($p < 0.05$) in total algal cells and organic matter in gut samples again occurred for heavily versus lightly infested individuals of both species. In addition, gut contents of individual *A. plicata* from one of two sites contained significantly less ($p < 0.05$) organic matter (0.92 versus 4.55 mg AFDW) and fewer algal cells (9.4×10^4 versus 2.3×10^5) than the combined gut contents of all zebra mussels (18-33 mm in length) attached to their shells. Gut analyses also revealed significant diet overlap between native unionids and infesting zebra mussels. Water samples collected from just above the mussel beds in 1997 showed that algal densities and total suspended solids at the heavily infested site (> 360 zebra mussels/m^2) were reduced by more than 50%, when compared to samples collected from the surface. Thus, competitive interactions or interference by zebra mussels likely reduced the availability of algal and detrital food resources for consumption by unionids.

Key words: algae, zebra mussels, unionids, Ohio River, competition

Since its introduction into Lake St. Clair, the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), has greatly reduced phytoplankton and bacterioplankton levels in the Great Lakes (Wu and Culver, 1991; MacIsaac *et al.*, 1992; Cotner *et al.*, 1995; Fanslow *et al.*, 1995; Heath *et al.*, 1995). Phytoplankton levels in Lake Erie, for example, dropped 62-92% (Leach, 1993), and planktonic diatoms decreased 85% despite sufficient nutrients for growth (Holland, 1993). Consequently, Secchi disk transparencies in Lake Erie have increased 85% (Leach, 1993). Phytoplankton grazing by zebra mussels also can alter the composition of the phytoplankton community. In Lake Huron, for example, zebra mussel feeding has shifted dominance from diatoms to filamentous green algae (Lowe and Pillsbury, 1995), and recent studies show selective rejection of the nuisance bluegreen alga *Microcystis* by zebra mussels, such that *Microcystis* becomes dominant in the plankton (H. Vanderploeg, NOAA, pers. comm.)

Zebra mussel colonization of the Great Lakes also has caused dramatic declines in the survival and fitness of native freshwater mussel populations (Hebert *et al.*, 1991; Hunter and Bailey, 1992; Haag *et al.*, 1993; Gillis and Mackie, 1994; Nalepa, 1994; Schloesser and Nalepa, 1994). By attaching to the shells of unionids, zebra mussels can directly affect unionid survival by interfering with feeding, respiration, balance, burrowing, and locomotion (Mackie, 1991; reviewed by Schloesser *et al.*, 1996). Large densities of zebra mussels, however, also can affect unionid survival indirectly by reducing or removing food resources from the water column (Lewandowski, 1976; Hebert *et al.*, 1991; Mackie, 1991; Haag *et al.*, 1993). A large gill-area to body-dry-weight ratio, and a large number of gill cirri in individual zebra mussels, allow for increased filtration efficiency and filtration rate relative to those of native unionids (Silverman *et al.*, 1995). Filtration rates of the freshwater mussel, *Lampsilis siliquoidea* (Barnes, 1823), for example, were found to be only one-tenth the filtration rate of individual zebra mussels (Heath *et al.*, 1995). In laboratory experiments, Baker and Hornbach (1997) reported that *Amblema plicata* (Say, 1817) filtered 74 ml/hr, while the 28 infesting zebra mussels filtered 130 ml/hr as a group. Thus, relatively small populations of zebra mussels can affect the feeding of unionids.

Zebra mussel populations in the lower Ohio River

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have achieved densities comparable to those in the Great Lakes (350,000/m²; A. Miller, USACOE, pers. comm.). Because of documented impacts to the phytoplankton communities and native mussel populations of the Great Lakes, large populations of zebra mussels in the Ohio River could have similar consequences for native mussel populations. Strayer and Smith (1996) found that low zebra mussel infestation rates in the Hudson River were associated with high unionid mortality and hypothesized that reduced food resources might be the cause. No studies, however, have directly confirmed whether zebra mussels affect the feeding of unionids in a riverine environment where organic materials are continually supplied from upstream. Thus, the objective of this study was to determine whether zebra mussels reduce unionid ingestion of phytoplankton and organic matter by (1) ingesting similar food resources, and (2) reducing food resources at the sediment-water interface.

METHODOLOGY

On 23 July 1996, ten specimens each of the threeridge, *Amblema plicata,* and the pimpleback, *Quadrula pustulosa* (I. Lea, 1831), were collected from a lightly infested site on the Ohio River near Parkersburg, West Virginia, which had a mean density of 0.3 zebra mussels/m², and a maximum of one zebra mussel/unionid (P. Morrison, USFWS, pers. comm.). On 16 August 1996, ten specimens of *A. plicata* were collected from a heavily infested site near Paducah, Kentucky, which had 3,600 zebra mussels/m² (A. Miller, USACOE, pers. comm.). Specimens of *Q. pustulosa* were difficult to find at this site, so on 21 August 1996, ten specimens were collected from another heavily infested site near Maysville, Kentucky, which had 360 zebra mussels/m² and a maximum of 92 zebra mussels/unionid (P. Morrison, USFWS, pers. comm.). In the field, mussel bodies were removed from shells, weighed, preserved in 95% ethanol, and transported to the laboratory for analysis.

In 1997, ten specimens each of *A. plicata* and *Q. pustulosa* were collected from a highly infested (370 zebra mussels/m²) and a lightly infested (< 1 zebra mussel/m²) site on the Ohio River for gut content analysis. In addition, all zebra mussels, 18-33 mm in length, attached to the shells of *A. plicata* were removed and preserved in 95% ethanol for gut content analysis. Zebra mussels 18-33 mm in length were chosen, because it is difficult to remove the entire gut contents of smaller individuals. At each collection site, water samples with algae were collected from the the surface and overlying the mussel bed, fixed with acid Lugol's solution (Saraceni and Ruggiu, 1969), and placed in settling chambers to compare the density and relative abundance of algal genera using inverted microscopes. Aliquots of 100 ml were then filtered through pre-ashed Whatman GF/F filters, dried (100°C), and ashed (500°C) to determine the ash-free dry weight (AFDW) of seston.

Gut contents of unionids and of zebra mussels attached to *Amblema plicata* were individually removed from each specimen, pooled, then suspended in 3 ml of water, and fixed with 50 µl of acid Lugol's solution for analysis. A 50 µl aliquot of the gut contents was placed on a microscope slide. Ocular grids divided the field of view into 59 transects, and algal cells were counted and identified to genus from two transects using an Ausjena/Nomarsky microscope at 400X. The variability of this semi-quantitative method (± 20%, $\alpha = 0.05$) was determined using ten counts from the same sample. The remaining gut contents were collected on pre-ashed Whatman GF/F filters, dried (100°C), and ashed in a muffle furnace (500°C) overnight to determine AFDW. Mean algal cell numbers and mean AFDW values in the gut samples of each species were compared by ANOVA.

RESULTS

In 1996, significant differences in total algal cells and organic matter were observed in guts of lightly and heavily infested unionids (Table 1). While mean algal cell numbers in guts of lightly and heavily infested *Amblema plicata* (5.7×10^5 versus 9.1×10^5 cells) were not significantly different ($p = 0.17$), the gut contents of lightly infested *A. plicata* had significantly more ($p < 0.01$) organic matter (4.6 mg AFDW) than heavily infested specimens (1.4 mg AFDW). Heavily infested *Quadrula pustulosa* showed significantly lower ($p < 0.05$) organic matter and mean algal cell number (0.6 mg AFDW and 1.8×10^4 cells, respectively) than lightly infested specimens (1.8 mg AFDW and 3.9×10^5 cells, respectively).

In 1997, significant reductions in organic matter content and total algal cells also were observed in guts of heavily infested unionids (Table 2). Organic matter content and total algal cells were significantly lower ($p < 0.05$) in the guts of heavily infested *Amblema plicata* (0.9 mg

Table 1. Mean algal cell number and ash-free dry weight (AFDW; ± SD) in guts of *Amblema plicata* and *Quadrula pustulosa* heavily infested (H) and lightly infested (L) with zebra mussels, July-August 1996.

Species	N	Algal Cell Number	AFDW (mg)
A. plicata (L)	11	$9.1 \times 10^5 \pm 6.0 \times 10^5$	4.6 ± 0.9
A. plicata (H)	10	$5.7 \times 10^5 \pm 4.9 \times 10^5$	1.4 ± 0.7
Q. pustulosa (L)	10	$3.9 \times 10^5 \pm 2.8 \times 10^5$	1.8 ± 1.0
Q. pustulosa (H)	10	$1.8 \times 10^4 \pm 9.2 \times 10^3$	0.6 ± 0.3

Table 2. Mean algal cell number and ash-free dry weight (AFDW; ± SD) in guts of *Dreissena polymorpha* and of *Amblema plicata* and *Quadrula pustulosa* heavily infested (H) and lightly infested (L) with zebra mussels, July-August 1997.

Species	N	Algal Cell Number	AFDW (mg)
A. plicata (L)	10	5.5 x 10^6 ± 2.4 x 10^6	5.1 ± 1.7
A. plicata (H)	10	9.4 x 10^4 ± 7.6 x 10^4	0.9 ± 0.8
Q. pustulosa (L)	10	1.9 x 10^6 ± 1.3 x 10^6	2.0 ± 1.2
Q. pustulosa (H)	9	6.9 x 10^4 ± 7.3 x 10^4	0.3 ± 0.2
D. polymorpha	9	2.3 x 10^5 ± 1.3 x 10^5	4.6 ± 3.6

AFDW and 9.4 x 10^4 cells, respectively) than lightly infested specimens (5.1 mg AFDW and 5.5 x 10^6 cells, respectively). Significant reductions in both organic matter content and total algal cells also were observed for heavily infested (0.3 mg AFDW and 6.9 x 10^4 cells, respectively) versus lightly infested (2.0 mg AFDW and 1.9 x 10^6 cells, respectively) *Quadrula pustulosa.*

Examination of unionid guts in 1996 and 1997 indicated that *Amblema plicata* and *Quadrula pustulosa* readily ingested a significant amount of detritus (ca. 90%) along with algal cells between 4 and 80 µm in length. Diatoms (Bacillariophyta) and green algae (Chlorophyta) dominated gut samples, and the dominant algal genera in 1996 (*Chlorella, Cyclotella, Navicula, Melosira,* and *Scenedesmus*; Table 3) and 1997 (*Chlorella, Cyclotella, Mougeotia, Melosira,* and *Scenedesmus*; Table 4) were similar. Usually, the relative abundance of algal genera within unionid guts was very similar to relative abundances of algae in water samples collected from the river bottom (Table 5). Interestingly, the pennate diatom *Synedra* dominated water samples at the lightly infested site (43%), but few if any of these > 100 µm-long cells were found in the unionid guts. In 1997, algal cell densities and seston AFDW at the water surface (8.37 x 10^4 cells/ml and 9.0 mg/l, respectively) and above the mussel bed (8.42 x 10^4 cells/ml and 9.0 mg/l, respectively) were nearly identical at the lightly infested site (Table 5). Seston AFDW (5.0 mg/l) and algal cell densities (2.2 x 10^4 cells/ml) overlying the mussel bed at the heavily infested site, however, were greatly reduced compared to samples taken from the surface (7.2 mg/l and 8.3 x 10^4 cells/ml, respectively).

In 1997, individual *Amblema plicata* collected from the heavily infested site were infested with > 100 zebra mussels/unionid, averaging 50 zebra mussels between 18 and 33 mm in length. Gut contents of zebra mussels contained large amounts of detritus. Pooled samples of zebra mussel guts contained five times more ($p < 0.05$) organic matter (4.55 mg versus 0.92 mg, respectively) and twice as many algal cells (2.3 x 10^5 cells versus 9.4 x 10^4 cells, respectively) than the specimen of *A. plicata* to which they were attached (Table 3). In addition, the dominant algal genera – *Chlorella, Cyclotella, Mougeotia, Melosira,* and *Scenedesmus* – and range of cell sizes (4-70 µm) in zebra mussel guts were nearly identical to those in infested *A. plicata* (Table 4). Relative abundances of algal genera within zebra mussel guts were similar to relative abundances in water samples collected from the river bottom (Table 5).

DISCUSSION

Reduced food resources at the sediment-water interface can cause decreased growth rates in bivalves despite adequate food resources at the water surface (Frechette and Bourget, 1985). This phenomenon could be particularly important for unionids in the lower Ohio River because zebra mussels occur in large densities at the sediment-water interface and often attach directly to the shells of unionids. In fact, water samples collected from just above the mussel beds in 1997 showed that algal densities and AFDW at the heavily infested site (> 360 zebra mussels/m^2) were reduced by more than 50% when compared to samples collected from the surface. Thus, it appears that zebra mussel densities in heavily infested sections of the Ohio River contribute to reduced food resources at the sediment-water interface.

Regardless of reductions in total algal cell densities, the effects of zebra mussel infestation on unionid ingestion can be reduced if zebra mussels and unionids selectively feed on different food types. Currently, studies on selective feeding in bivalves appear to be inconclusive. Some authors have concluded that bivalves select food particles of high quality (Allen, 1914; Loosanoff and Engel, 1947; Shumway *et al.*, 1985), while others have concluded that feeding is non-selective (Churchill and Lewis, 1924; Gale and Lowe, 1971; Bayne *et al.*, 1976). Thus, selective feeding could be species dependent. Zebra mussels have been reported to ingest a wide range of food particles between 0.7 and 450 µm (Mikheyev, 1967; Jorgensen *et al.*, 1984). However, Sprung and Rose (1988) indicated that retention efficiency in zebra mussels is maximized for food particles between 5-35 µm; Ten Winkle and Davids (1982) also concluded from gut content analysis that zebra mussels select particles between 15-50 µm. In our study, zebra mussel gut samples contained food particles between 4-70 µm in maximum dimension. In comparison, Miura and Yamashiro (1990) indicated that the unionid *Anodonta calipygos* (Kobelt, 1879) ingests food particles between 0.5 and 100 µm. Like for the zebra mussel, maximum retention efficiencies were

Table 3. Percent relative abundances (SD) of algae in guts of lightly (L) and heavily (H) infested *Amblema plicata* and *Quadrula pustulosa* collected from the Ohio River, July-August 1996. (+, presence [≤ 2%]; - , absence).

Algae	*A. plicata* (L)	*A. plicata* (H)	*Q. pustulosa* (L)	*Q. pustulosa* (H)
Chlorophyta				
Ankistrodesmus	–	–	+	–
Chlamydomonas	+	–	+	–
Chlorella	15.3 (8.7)	15.0 (11.2)	42.3 (18.8)	63.0 (29.4)
Chlorococcum	+	+	+	–
Closterium	–	–	+	–
Coelastrum	–	–	+	–
Cosmarium	–	–	+	–
Gonium	–	+	–	–
Mougeotia	+	+	+	–
Oedogonium	–	+	–	–
Oocystis	+	–	–	–
Pediastrum	–	+	–	–
Scenedesmus	6.2 (5.9)	5.2 (4.7)	6.6 (5.8)	–
Schroederia	+	–	–	–
Selenastrum	–	–	+	–
Spirogyra	+	–	–	–
Staurastrum	+	–	–	–
Bacillariophyta				
Achnanthes	+	–	+	–
Cocconeis	+	–	+	–
Coscinodiscus	+	+	+	–
Cyclotella	11.8 (2.7)	45.1 (11.1)	7.0 (3.8)	31.9 (30.8)
Cymbella	+	+	+	–
Diatoma	+	–	+	–
Fragilaria	–	–	+	–
Gomphonema	+	–	+	–
Melosira	5.7 (4.0)	16.0 (11.0)	8.8 (8.2)	2.8 (6.4)
Navicula	26.5 (7.4)	2.2 (0.6)	10.6 (6.2)	
Nitzschia	–	–	+	–
Pinnularia	+	+	+	–
Pleurosigma	–	–	+	–
Stephanodiscus	–	+	–	–
Surirella	+	–	–	–
Synedra	+	+	+	–
Tabellaria	+	–	–	–
Cyanoprokaryota				
Chroococcus	+	+	+	–
Merismopedia	–	+	–	–
Oscillatoria	+	–	+	–
Spirulina	–	–	+	–
Other				
Chromulina	–	+	–	–
Dinobryon	+	–	–	–
Peridinium	–	+	+	–
Chroomonas	+	–	–	–

found for intermediate-sized particles (5-30 µm). Maximum filtration rates for the unionid *Elliptio complanata* (Lightfoot, 1786) also were found for particles between 4 and 5 µm (Paterson, 1984). In our study, unionid gut analyses indicate that unionids ingest food particles between 4 and 80 µm in maximum dimension. Thus, zebra mussels and unionids in the Ohio River ingest food resources of similar size.

Relative abundances of algal genera ingested by zebra mussels and the two unionid species were very similar to those in water samples collected immediately above the mussel bed, giving no evidence of selective feeding. The only exception was the pennate diatom *Synedra* which was not readily ingested by unionids or zebra mussels,

Table 4. Percent relative abundances (SD) of algae in guts of *Dreissena polymorpha* and lightly (L) and heavily (H) infested *Amblema plicata* and *Quadrula pustulosa* collected from the Ohio River, July-August 1997. (+, presence [≤ 2%]; –, absence).

Algae	*A. plicata* (L)	*A. plicata* (H)	*Q. pustulosa* (L)	*Q. pustulosa* (H)	*D. polymorpha*
Chlorophyta					
Ankistrodesmus	–	–	+	–	–
Chlamydomonas	–	+	+	+	+
Chlorella	1.7 (1.4)	13.8 (10.1)	9.6 (8.8)	30.8 (24.7)	37.5 (16.8)
Chlorococcum	+	+	+	+	+
Cosmarium	–	–	+	–	–
Crucigenia	–	–	+	–	–
Gonium	–	–	+	–	–
Mougeotia	40.6 (7.5)	5.1 (7.0)	21.2 (11.7)	5.3 (9.3)	14.2 (12.2)
Oedogonium	+	–	–	–	–
Pandorina	+	–	+	–	–
Pediastrum	+	+	+	+	+
Scenedesmus	9.5 (3.6)	5.7 (2.0)	8.2 (5.9)	10.9 (9.6)	13.9 (10.7)
Trebouxia	+	–	–	–	–
Bacillariophyta					
Coscinodiscus	–	–	+	–	–
Cocconeis	–	–	+	–	–
Cyclotella	21.3 (11.9)	38.5 (18.6)	25.9 (6.9)	16.2 (12.7)	5.4 (4.5)
Diatoma	+	–	+	–	–
Gomphonema	+	–	–	–	–
Melosira	13.7 (9.2)	25.9 (16.7)	17.6 (9.4)	28.5 (18.3)	13.7 (10.4)
Navicula	+	+	+	–	+
Stephanodiscus	+	–	+	–	–
Synedra	–	–	+	–	–
Tabellaria	–	–	+	–	+
Cyanoprokaryota					
Aphanocapsa	–	–	+	–	–
Chroococcus	+	–	+	–	–
Oscillatoria	–	–	+	–	–
Other					
Peridinium	–	–	+	–	–

probably because of its cell length of > 100 µm. In unionid gut samples, *Mougeotia*, *Cyclotella*, and *Melosira* did appear in slightly greater abundance than in water samples. Regardless of selective feeding, unionid and zebra mussel gut contents contained a nearly identical assemblage of dominant algal genera. Thus, by ingesting food particles of similar size and type, zebra mussels in the Ohio River can compete directly with native unionids for food resources. However, competition can only be confirmed if the food items measured to assess diet overlap constitute a significant portion of the total diet of one of the potential competitors (Buss and Jackson, 1981). Unfortunately, there are no studies on the dietary or nutritional requirements of freshwater mussels.

Significant reductions in the mean AFDW and total algal cell number from gut samples of heavily infested versus lightly infested native freshwater mussels indicate that interference competition for food resources is occurring in the lower Ohio River. Since the arrival of the zebra mussel, unionid mortality thus far at various sites in the lower Ohio River has been estimated at 20-40% (P. Morrison, USFWS, pers. comm.). Interference with unionid feeding by zebra mussels, however, also could have long term consequences for the overall fitness of native mussels. Recent studies have shown that native unionids from the heavily infested Ohio River have significantly reduced glycogen levels relative to unionids from lightly infested areas (Patterson *et al.*, 1997). Glycogen is an important energy reserve for animals, especially bivalves (de Zwann and Zandee, 1972; Barber and Blake, 1981; Bayne and Newell, 1983; Haag *et al.*, 1993), and significant reductions can lead to chronic mortality or declines in reproductive success. Gonad development in marine bivalves, for example, has been shown to continue despite reduced energy reserves (Gabbott and Bayne, 1973; Bayne, 1975), but subsequent growth rates and energy reserves of the developing larvae decreased (Bayne, 1972; Helm *et al.*, 1973; Bayne *et al.*, 1975). Thus, by reducing food resources and interfering

Table 5. Percent relative abundances of algae at the surface and just above the mussel bed in the heavily (H) and lightly infested (L) Ohio River, July-August 1997. (+, presence [≤ 2%]; –, absence).

Algae	Surface (L)	Bottom (L)	Surface (H)	Bottom (H)
Chlorophyta				
Ankistrodesmus	–	+	–	–
Chlamydomonas	6	5	16	11
Chlorella	10	8	25	23
Chlorococcum	+	2	+	7
Gonium	–	+	–	–
Mougeotia	8	2	6	5
Oedogonium	+	+	–	+
Pediastrum	+	+	–	+
Scenedesmus	12	16	13	12
Staurastrum	+	+	+	–
Bacilariophyta				
Coscinodiscus	–	+	+	+
Cyclotella	4	5	3	13
Diatoma	+	+	–	+
Melosira	+	2	4	5
Navicula	–	+	–	+
Stephanodiscus	+	+	–	+
Synedra	12	43	11	10
Cyanoprokaryota				
Chroococcus	–	2	+	7
Merismopedia	8	7	–	–
Oscillatoria	+	6	3	3
Other				
Mallomonas	–	–	–	+

with normal feeding, zebra mussels could have their greatest effect on the long-term persistence of unionid beds in the lower Ohio River through reduction in reproductive success and recruitment.

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Research Note: A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed, or dried material

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Abstract: A new method for cleaning tissue from gastropod radulae is presented that is non-destructive compared to the methods presently in use. Present methods are time sensitive because the caustic solutions used can damage radula. This new method uses a detergent and Proteinase K enzyme which are not time sensitive. This method will work with frozen, alcohol-fixed, or dried material but not with formalin-fixed material. In addition, the tissue supernatant can be used for subsequent standard DNA-extraction techniques.

Key words: gastropod, snail, radula, cleaning method, preparation

The present methods for cleaning gastropod radulae for viewing use either sodium hypochlorite (NaOCl) or potassium hydroxide (KOH). Because of the caustic nature of these chemicals, careful monitoring of the specimen is required to avoid damaged or dissolved radulae. An alternative method routinely used to digest tissue for DNA extraction (Sambrook *et al.*, 1989) has proven to be as effective in cleaning radulae and avoids the likelihood of unwanted erosion of the specimens. This method is effective for preparing radulae from frozen, alcohol-fixed, newly dried, and from dried specimens collected over 30 years ago. This method has not proven to be useful for cleaning radulae from specimens preserved in formalin because of the protein cross-linking that occurs with this method of fixation.

MATERIALS

The necessary equipment and stock solutions required are listed in Table 1. In addition, the following lab equipment is needed: dissecting microscope, microforceps, scalpel, and pipettes. Additional useful tools include: insect pins (very good probes) and sharpened applicator sticks (excellent tools for manipulating the radula during mounting). Cleaned radulae for scanning electron microscopy (Fig. 1) were sputter-coated with gold-palladium and scanned on a Hitachi S-2500 scanning electron microscope at the University of Alabama.

THE METHOD

With freshly frozen material the gastropod is generally partially extended so that it is a simple matter to extract the animal from its shell by grasping the foot and body behind the head and gently pulling. Typically in alcohol-fixed and/or dried material, the animal has withdrawn into the shell. Removal of the snail body from the shell can be accomplished by carefully cracking the shell. After separating the head from the rest of the animal, rinse the head with water to remove any extraneous material that might have adhered to it (*i. e.* shell fragments). Place each specimen in an individual 1.5 ml microcentrifuge tube and add 500 µl NET buffer and 10 µl Proteinase K (Table 1). Close the tube and tape it to the mixer platform, and place the mixer an incubator at 37°C. With fresh or alcohol-fixed material, cleaning should take about 2-3 hr. Dried tissue specimens can require 2-4 d, depending on the amount of starting material. For dry material it may be necessary to replace the NET buffer and Proteinase K after 24-48 hr. Once cleaning is complete, as determined by examination under a dissecting microscope, remove the radula and rinse in several changes of deionized water to removed all traces of the NET buffer. The remaining supernatant can be used to obtain DNA using DNA standard phenol/chloroform extraction techniques. The radula is then stored in 25% ethanol until ready for mounting. At this point the clean radula can be mounted by a procedure appropriate for the type of visualization to be used. Some cleaned radulae have

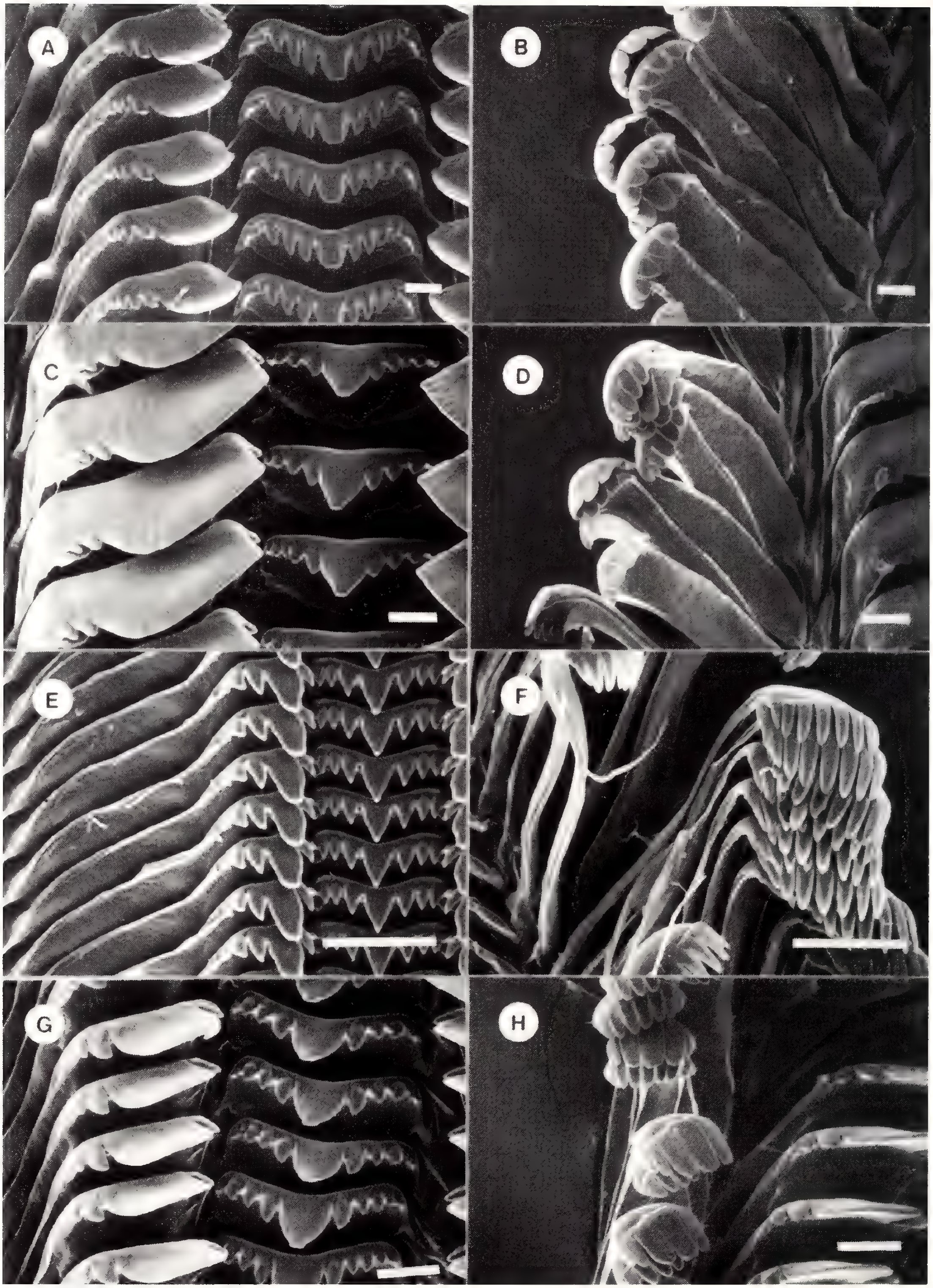

Fig. 1. Examples of radulae cleaned by the described method. A-B. *Io fluvialis* (Say, 1825). C-D. *Leptoxis (Athearnia) crassa anthonyi* (Redfield. 1854). E-F. *Terebia granifera* (Lamarck, 1822). G-H. *Gyrotoma pyramidatum* Shuttleworth, 1845. The radulae were prepared from the following types of tissue: fresh frozen (A-B); alcohol fixed (C-D); alcohol fixed and dried; (E-F); 30-year old unfixed dried material (G-H). Figs. A, C, E, and G show the central and left lateral tooth; B, D, and H show the left pair of inner and outer marginal teeth; and F shows the right pair of inner and outer marginal teeth. Scale bars = 50 μm.

been stored in 25% ethanol for up to 6 mo before mounting and no degradation was observed.

The radula and the radular ribbon do not appear to be affected by the enzymatic action of Proteinase K as evidenced by scanning electron micrographs (Fig. 1). Therefore in this cleaning method careful monitoring is not necessary. An additional benefit of this procedure is that the radular ribbon from dried material is rehydrated and becomes flexible enough to undergo the rigors of mounting.

Table 1. Equipment and stock solutions.

Incubator
Platform mixer
1.5 ml microcentrifuge tubes

NET buffer
  1 ml Tris pH 8.0
  2 ml 0.5 M ethylene diamine tetraacetic acid (EDTA)
  1ml 5 M NaCl
  20 ml 10% sodium dodecyl sulfate (SDS) (Sigma-Aldrich Chemical Company no. L4522)
  76 ml deionized water

Proteinase K stock*
  20 mg Proteinase K* (Sigma-Aldrich Chemical Company no. P4914)
  1 ml deionized water

  * store at -20 °C

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Research Note: In search of *Rossia pacifica diegensis* S. S. Berry, 1912

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Abstract: We describe the eggs of *Rossia pacifica* S. S. Berry, 1911, from a depth of 1,200 m off of southern California. The large size of the eggs and the great depth and low temperature from which they were retrieved contrasts strongly with smaller eggs of this species taken in shallower, warmer water along the western coast of the contiguous United States. This raises questions as to whether more than one taxon in the genus *Rossia* is present in this area.

Key words: Cephalopoda, Rossiidae, larvae, eggs, subspecies

S. S. Berry first described the sepiolid cephalopod *Rossia pacifica* from Alaskan waters in 1911. In 1912, he provided a more complete description based on specimens taken from southern Alaska to southern California. His specimens were trawled from depths of at least 30 to 310 m, possibly as deep as 550 m. In recent years, the known habitat of the species has been extended through the Bering Sea to the Tsushima Straits between Korea and southern Japan (Nesis, 1987), and southward from off of the southern California coast to about 28°N off of Baja California (F. G. Hochberg, pers. comm.); the known depth range remains approximately the same.

Considerable information now exists on the biology of *Rossia pacifica* (Brocco, 1971; Summers, 1985; Anderson, 1987, 1991; Summers and Colvin, 1989; Anderson and Vanderwerff, 1989; Anderson and Shimek 1994). Off of the northwestern coast of the continental United States, it spawns clusters of white eggs with each egg about 1 cm in diameter and ovoid in shape, except for a flattened area where the eggs are cemented to the substratum (Anderson and Shimek, 1994). The eggs have a tough outer coating and a papilla that lies opposite the flat attachment site (Anderson, 1991). Off Washington state, scuba divers have observed egg clusters attached to rocks at ocean depths of 18 to 30 m (Anderson and Shimek, 1994), and eggs have been spawned and reared in local laboratories and public aquaria (Summers, 1985; Anderson, 1991). Eggs have developed at 10°C in a temperature-controlled aquarium and from 6-12°C (monthly means) in a running seawater system that reflected ambient ocean temperatures. Under both regimes, the minimum hatching times were four and three-quarters months with spontaneous hatching continuing for an additional two months (Summers, 1985). At hatching, the young measured 6-8 mm mantle length (ML) (Summers, 1985; Anderson, 1991). Recently, Shevtsov and Radchenko (1997) reported *R. pacifica* eggs with developing embryos from the Bering Sea at a depth of 250 m and a temperature of 1.6°C. In the northern Japan Sea, the distribution and spawning of *R. pacifica* has been discussed by Shevtsov and Mokrin (1997).

In his 1912 paper, Berry noted that specimens of *Rossia pacifica* caught off of San Diego, California, were captured at greater depths than in other localities and differed morphologically. Specifically, they (1) were smaller and more slender and delicate, (2) had relatively larger fins, and (3) carried suckers on the arms in predominantly two, rather than four, rows. Berry (1912) designated these specimens as a new subspecies, *R. p. diegensis*, while apparently feeling that the morphological differences were sufficient for specific status. His type specimens are presently deposited in the U. S. National Museum of Natural History (Washington, DC; (USNM 214376). However, as these specimens were geographically separated from other Californian specimens found in Monterey Bay, he believed intergrades might eventually be found that would negate specific status. The possible validity of this taxon has been virtually ignored since his description.

MATERIAL AND METHODS

The material examined was collected by the R/V NEW HORIZON, 13 December 1995, with a 25 ft (7.6 m) semiballoon trawl at 33°10.25'N, 118°25.2'W (tow mid-point) in the Santa Catalina Basin, California, at a depth of 1,204-1,222 m. The bottom temperature at this locality was 4.1°C. The trawl had an outer mesh of 3.8 cm (stretch) and an inner mesh of 1.3 cm (stretch). The bottom in this area is a silty clay with a disaggregated median grain size of 4.0 μm and the benthic macrofaunal community is heavily dominated by deposit-feeding polychaetes (Kukert and Smith, 1992). The specimens are deposited in the Santa Barbara Museum of Natural History.

RESULTS

Three large cephalopod eggs were retrieved from the trawl. Two measured 18 x 16 mm, one of which is figured (Fig. 1A). The third was 14 mm in one dimension; damage prevented measurement in the other axis. The two larger eggs had well-developed embryos; the smaller egg had a badly malformed embryo. The surface of the egg capsules had been somewhat abraded by the trawl, preventing description other than to note that a papilla was present and that the capsule wall was about 0.5 mm thick and transparent (Fig. 1B). No obvious flat attachment site was found on the capsule.

The developing embryos appeared to be at roughly the same stage of development. One was dissected out. It has a mantle length of 2 mm at about Naef's stage XVII or XVIII (Naef, 1928) and the size of the external yolk sac, not measured due to fracturing, dwarfs the size of the embryo (Fig. 1B). Large fins arise from the sides of the mantle rather than from the dorsal side (Fig. 1C). Each fin is nearly semicircular in outline and subterminal in posi-

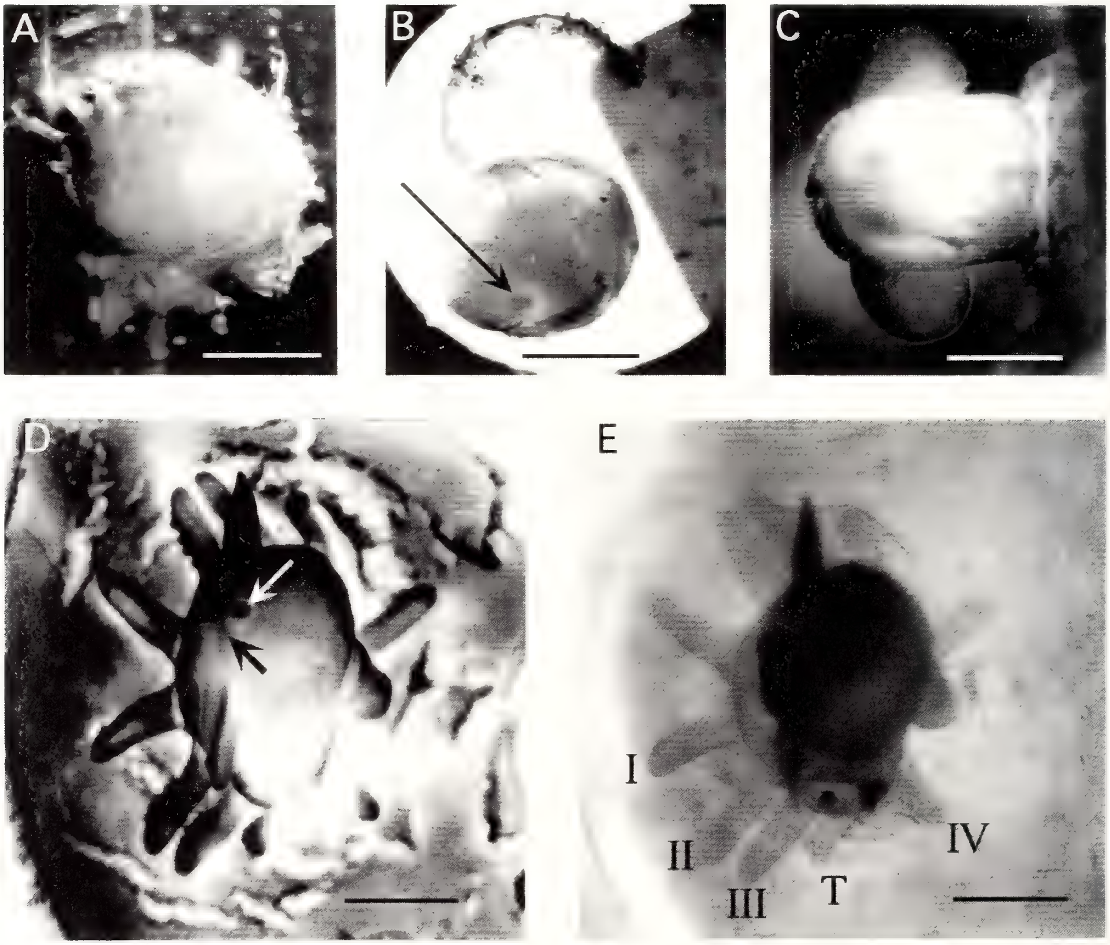

Fig. 1. *Rossia* eggs and embryos. A. Single egg in outer capsule. Photographed with reflected light. B. Single egg with embryo (arrow) and portion of outer capsule separated from egg. Distance between two lines on label paper is 1 cm. Photographed with transmitted light. C. Dorsal view of embryo showing fin shape, free dorsal mantle margin, and terminal spine. Photographed with reflected light. D. Posterior view of stained (methylene blue) embryo. White arrow indicates terminal spine; black arrow indicates hatching organ. Photographed with reflected light. E. Posterior view of unstained embryo. Photographed with reflected light. Scale bars = 1 cm (A, B), 1 mm (C, D, E). (I-IV, left arms; T = tentacle).

tion, the dorsal mantle margin is not fused with the head (Fig. 1C), and the hatching organ and terminal spine are easily seen (Figs. 1C, D). The embryo extends at right angles to the yolk sac with the arms spread laterally over the surface of the yolk (Figs. 1D, E). It has eight arms and two tentacles (Figs. 1D, E), with two rows of developing suckers on arms II and III; the armature of the other arms was not examined.

DISCUSSION

Although the embryonic development of *Rossia pacifica* has not been described, our embryos can be identified on the following basis. They can be placed in the Decapodiformes by the presence of eight arms and two tentacles, and in Sepiolidae by the (1) stubby, blunt shape of the mantle, (2) position and shape of the fins, and (3) presence of a terminal spine (Boletzky, 1991). Within the Sepiolidae, its identity is further narrowed by the lack of a dorsal head-mantle fusion. Within the North Pacific Ocean, the only sepiolids lacking head-mantle fusion are Rossia and *Heteroteuthis*, but only *Rossia* is presently known from Californian waters. In addition, the small, pelagic *Heteroteuthis* hatches at less than 2 mm ML (Boletzky, 1978) and can be eliminated by the large size of our eggs. By process of elimination, therefore, the eggs belong to a species of *Rossia*.

To our knowledge, the only other record of rossiian eggs from this depth is that of *Neorossia caroli* (Joubin, 1902) which was taken in a trawl at 1,234 m in the Mediterranean Sea (Villaneueva, 1992). This species, known to reach depths of 1,744 m (Villaneueva, 1992), could be the deepest living of all rossiian species. The eggs described here are also very large for a member of Rossiinae as this group typically has eggs of 5-7 mm in diameter (Lu *et al.*, 1992). Judging from the known ratio of egg size to hatchling size in shallow-water *Rossia pacifica*, an embryo hatching from an egg of 18 mm should be more than 10 mm ML.

The only species of *Rossia* known from Californian waters is *R. pacifica*. The eggs described here differ from the *R. pacifica* eggs taken off of the northwestern coast of the continental United States in that they are much larger and were found at greater depths and at lower temperatures. Either *R. pacifica* exhibits great variability in these parameters or another *Rossia*, possibly *R. p. diegensis*, is present. On the other hand, *R. pacifica* could be a cold-water, large-egged form and the second taxon could be an unnamed warmer-water, small-egged form found at rather shallow depths along the western coast of the contiguous United States. In the latter case, *R. p. diegensis* would be invalid. We strongly urge researchers to reexamine the possibility that a second taxon exists.

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SYMPOSIUM: TRADITIONAL VERSUS PHYLOGENETIC SYSTEMATICS OF MOLLUSKS

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Reconciling observed patterns of temporal occurrence with cladistic hypotheses of phylogenetic relationship

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Abstract: Cladistic analyses of well-sampled groups with a complete and abundant fossil record can yield phylogenetic hypotheses that conflict with stratigraphic data, even to the extent of supporting phylogenies that appear to invert the stratigraphy. This is most probably due to the convergent evolution of similar morphologies (*i. e.* homoplasy), rather than the inadequacy of the fossil record. Several ways have been proposed to resolve this problem (stratophenetics, stratocladistics, character refinement, etc.). This paper proposes an iteractive technique to construct a tree more consistent with the observed fossil record by building separate phylogenies for different stratigraphic intervals which can then be assembled into a composite phylogeny. Columbellid gastropods of the genus *Strombina* Mörch, 1852, were used to test this approach. *Strombina* originated and diversified in the Caribbean during the Miocene and Pliocene. During the Pliocene, they became nearly extinct in the Caribbean, but diversified in the eastern Pacific. Phylogenies of 42 species based only on shell morphology (49 characters, 186 states) yielded trees with high stratigraphic inconsistency and ghost lineages that require the presence of descendants 10 million years or more before the first appearance of their hypothesized ancestors. Removal of species that originated after the Pliocene resolved most of these stratigraphic inconsistencies although some ghost lineages remained. This Miocene/Pliocene tree was then used to root the trees illustrating the relationships among Pleistocene/Holocene species. This final composite tree is more consistent with the hypothesized fossil record for the group than the original tree.

Key words: phylogeny, fossil record, consistency, morphology, gastropods, *Strombina*

Most analyses of extant taxa exclude fossil data with the justification that the fossil record is too incomplete and too poorly dated to be useful (Hennig, 1966, 1981; Nelson, 1989; Patterson, 1981; Goodman, 1989). Although this is true for many taxa without durable skeletons, many marine invertebrate taxa such as mollusks, bryozoans, and corals have a rich fossil record. Moreover, fossil collections of these taxa can greatly outnumber collections of extant species. Also, the ages of many fossil collections are known within a range of 1-2 million years, and often better, which is adequate to order occurrences over the typically 20 million years of history of most genera (Cheetham, 1987; Budd, 1989; Budd *et al.*, 1994, 1996). This can depend on the density of sampling (*i. e.* number of samples and sampling interval), but even in studies of taxa with a sparse fossil record the inclusion of fossils can help discriminate morphospecies and increase the consistency of cladistic hypotheses, thus providing a more effective means of rooting trees than comparison with a living outgroup (Cheetham and Hayek, 1988; Jackson and Cheetham, 1994; Cheetham and Jackson, 1995; Marshall, 1995).

Fossils can provide reliable estimates of the order in which lineages appear in the history of life (Gardiner, 1982; Gauthier *et al.*, 1988; Norell and Novacek, 1992a, b; Novacek, 1992a; Foote, 1996), thus providing a temporal component to calibrate phylogenetic hypotheses. However, the fossil record is often incongruent with cladistic topologies based on morphology alone. There are two sources of disagreement: (a) when the cladistic results require the presence of long ghost lineages that conflict with a rich fossil record for the time interval of interest (Cheetham and Hayek, 1988; Jackson and Cheetham, 1990, 1994; Wagner, 1995; Wagner and Erwin, 1995); or (b) when ancestor-descendant relationships are turned upside-down relative to their stratigraphic sequence (Jackson and Cheetham, 1994). This disagreement can be by as much as 5-10 million years. Such strong conflict between a good fossil record and a given cladistic hypothesis suggests that morphological convergence (*i. e.* homoplasy) could be responsible for putative relationships within the branches of the cladograms (Campbell and Barwick, 1988, 1990; Allmon, 1989; Bodenbender, 1994).

The fossil record can be employed in different ways to try to resolve this disagreement. For example, extinct taxa can be used to infer character polarity (Donoghue *et al.*, 1989; Novacek, 1992b). The fit between stratigraphic information and trees can be used to choose among most parsimonious trees based on morphological data alone (Norell, 1993; Huelsenbeck, 1994; Suter, 1994; Benton and Storrs, 1994, 1996). Stratocladistics (Fisher, 1980, 1988, 1991, 1992, 1994; Clyde, 1994; Clyde and Fisher, 1997) uses stratigraphic information to calculate the "stratigraphic parsimony debt" - a measure of the discrepancies between expected and observed order of stratigraphic occurrences. Although in this case both types of data are allowed to interact at the same level, morphological data are still assigned more weight, thus biasing the output. Common to many of these studies is the tendency to test the adequacy and completeness of the fossil data by quantifying the congruence between stratigraphic information and phylogenetic hypothesis based on morphological data (Marshall, 1990, 1994, 1997; Huelsenbeck, 1994; Benton and Hitchin, 1996; Huelsenbeck and Rannala, 1997). Several authors (Gingerich, 1979; 1994; Cheetham, 1987; Budd, 1988; Stanley *et al.*, 1988; Geary, 1990; Wei, 1994) have advocated the use of stratophenetics, a clustering technique, to reconstruct relationships using both Recent and fossil species, using overall morphologic similarity to connect populations through time. Moreover, stratophenograms give excellent results for groups with a very complete, well-preserved fossil record, like foraminiferans, cheilostome bryozoans, some gastropod groups, and small vertebrates.

Another way of using the fossil record is to construct separate analyses of subsets of a clade between major pulses of evolution (Budd and Coates, 1992). The justification for this method is the assumption that convergent taxa tend to originate during such pulses or turnovers. These separate analyses can then be used to assemble a composite tree across the pulse by building on the tree for the previous interval, such that later taxa cannot totally change hypothesized relationships among much older taxa. This method assumes that observed stratigraphic ranges correspond closely to "true" stratigraphic ranges and that members of different but contemporary clades had approximately the same probabilities of preservation and recovery. Consequently, it considers the possibility that the phylogenetic hypothesis could be incorrect, rather than blaming the fossil record for its deficiency. The aim of this paper it is to present a case study where this methodology was used to choose among competing hypotheses of relationships. The final accepted tree is one that better reconciles observed and expected patterns of temporal occurrences for the group in question. This methodology applies only to species-level analyses, differing in this from stratocladistics, which can be used in cases of uncertain specific affinities and also with supraspecific taxa.

MATERIALS AND METHODS

TAXA AND CHARACTERS

The Isthmus of Panama provides a well-documented example of a turnover event that occurred approximately 3-2 Ma (Allmon *et al.*, 1993, 1996; Jackson *et al.*, 1993, 1996; Budd *et al.*, 1996; Cheetham and Jackson, 1996; Fortunato and Jackson, 1996). The large molluscan fossil collections now available for the Neogene to Recent of tropical America, with greatly improved geochronological data (Coates *et al.*, 1992; Collins and Coates, 1993; Coates and Obando, 1996; A. G. Coates, unpub.) and from tens to hundreds of localities, contain thousands of specimens belonging to several highly diverse clades (J. B. C. Jackson *et al.*, unpub.). They permit a look at evolution at the species level and test the hypothesis that homoplasy arises during mass extinction/origination events.

The taxon used in this study is a group of small buccinoidean gastropods, mostly 1-5 cm long, the so-called *Strombina*-group (Jung, 1986, 1989). This group comprises approximately 110 species ranging from the early Miocene (about 20 Ma) through the Holocene, with an excellent fossil record and several thousand specimens collected. Their alpha taxonomy has been revised recently (Jung, 1989), and is assumed to be correct (or correctable with little effort) for this study. The wide distribution of the group, both in space and time, is an important aspect for the technique used here. Species diversity slowly increased over time, mainly in the Caribbean. In the eastern Pacific there were always many fewer species until approximately 1.8 Ma, at the Plio-Pleistocene boundary, when there was a sudden geographic shift in diversity from the Caribbean to the eastern Pacific (Jackson *et al.*, 1993). The fact that there are fewer collections (and specimens) from the eastern Pacific than from the Caribbean sea could affect the observed diversity pattern through time. Nevertheless, in spite of being very abundant, most taxa of the *Strombina*-group occur only in one region and only during one time interval, which, given the extensive records overall, cannot be merely a sampling artifact.

Of the five genera that comprise the *Strombina*-group, only the genus *Strombina* Mörch, 1852, was used for this study. This is the most abundant and diverse genus in the group, with 42 species, of which 20 originated during the last three million years. Previous results based in both shell morphology and anatomy strongly support the monophyly of this genus (Fortunato and Jung, 1995).

The material analyzed here (Table 1) consisted of all available fossil and Holocene collections from more than 20 institutions and private collections used by Jung (1989) for his taxonomic revision of the genus *Strombina*. These collections were supplemented by the fossil collections from the Panama Paleontological Project (PPP), maintained in the Naturhistorisches Museum, Basel, Switzerland.

The characters used in this study were selected from a larger suite of characters, including both measurements

Table 1. Number of lots and specimens used in this study.

Subgenus	Lots	Specimens
Strombina	362	7085
Spiralta	459	2646
Lirastrombina	291	1363
Arayina	11	117
Recurvina	310	2969
Costangula	98	603

(continuous) and qualitative (discrete) characters of shell morphology and anatomy, as defined in a previous work to test the monophyly of the *Strombina*-group (Fortunato and Jung, 1995). Only shell morphology was considered here to allow equal evaluation of both extant and fossil species. Of these 49 characters, six are measurements of overall shell and aperture dimensions; 38 are qualitative (discrete) characters related to various aspects of shell ornamentation and armor, as well as aperture armor; and five are composite characters referring to overall shell size, shape, and spire, as well as apertural size and shape. All characters were unordered and equally weighted. Unknown states were coded as missing. Continuous characters were coded using the Duncan's procedure for multiple-comparison tests of species means (Winer, 1971). Detailed descriptions of these characters, as well as coding procedures and the data matrix, will be published elsewhere.

ANALYTIC PROCEDURES

Species-level cladistic phylogenies for 42 *Strombina* species were generated using Hennig86 version 1.5 (Farris, 1988). Trees were rooted on the three oldest species of the genus *Sincola* Olsson & Harbison, 1953, which is the second oldest genus of the *Strombina*-group. Both *Sincola* and *Strombina* first occur in the proto-Caribbean region approximately 18 million years ago. Heuristic searches were performed using the "mhennig" followed by "bb" options to generate trees. Exhaustive searches were impossible to carry out because of the excessive amounts of time required. No consensus trees were requested; all most-parsimonious trees obtained in each analysis were evaluated and only the trees that were congruent with the time of origination and extinction hypothesized for the group in question were retained. The single tree that was most consistent with the observed fossil record was then used for the reconstructions.

Table 2 summarizes the sequence of procedures and results for all four sets of cladistic analyses. All assumptions were the same for the four sets of analyses, only the species included varied. For Analysis 1, all 42 species rooted on three *Sincola* species were used regardless of stratigraphic level. Evaluation of the most parsimonious trees yielded by this analysis (the "Total" tree; Fig. 1) showed that taxa from different stratigraphic levels were evenly scattered across the trees, indicating extremely high stratigraphic inconsistency. The taxa were therefore subdivided into two groups, pre- and post-turnover, depending on their time of origination. Analysis 2 was run using only the 22 species that originated before the turnover event that took place in tropical America ("pre-turnover" species; origination times older than three million years). The trees yielded by this analysis (the "Mio-Pliocene" tree; Fig. 2) were evaluated and taken as a fixed point for further work. Analysis 3 was subdivided into two phases, each consisting of a

Table 2. Sequence of procedures used in each analysis and the results obtained.

	Analysis 1	Analysis 2	Analysis 3A	Analysis 3B	Analysis 4
Input:	42 ingroup taxa 3 outgroup taxa 49 characters	22 ingroup taxa older than 3 Ma 3 outgroup taxa 49 characters	11 Mio-Pliocene ingroup taxa (left clade) 3 outgroup taxa 49 characters	9 Mio-Pliocene group taxa (right clade) 3 outgroup taxa 49 characters	All trees
Procedures:	Morphologic parsimony	Morphologic parsimony Removal of taxa younger than 3 Ma	Morphologic parsimony Break between taxa 28/27 Include oldest *Strombina* (taxon 4) Add younger taxa to each lineage	Morphologic parsimony Break between taxa 28/27 Include oldest *Strombina* (taxon 4) Add younger taxa to each lineage	Connect each tree sequentially following stratigraphic order Retain main clades delineated in Analysis 1
Result:	"Total tree" 2 main clades Stratigraphic inconsistency	"Mio-Pliocene tree" 2 main clades Stratigraphic consistency	Final tree for *Strombina/ Recurvina* clade	Final tree for *Lirastombina/ Spiralta/Costangula* clade	Composite tree Stratigraphic consistency

series of separate cladistic analyses including the younger species which originated after the turnover event (the "post-turnover" species; origination times younger than three million years). In each of these phases, the extant species were added to each subclade as defined by the "Mio-Pliocene" tree and supported by the "Total" tree. The results of the third set of analyses were then used to build a composite tree (Analysis 4) based on the "Mio-Pliocene" tree and with all of the extant species added. Ancestor-descendant relationships were interpreted for each of these subclades based on the results of Analyses 2 and 3. The taxon of the same subgenus which lay at the tip of the "Mio-Pliocene" tree was considered to be the ancestor of the subsequent Holocene species. The trees yielded by Analysis 3 were attached to these ancestor taxa.

In another words, all analyses were performed as though a systematist decided to test for homoplasy three million years ago, before the major turnover event took place in Central America. One of the assumptions is that the relationships that existed then could not be changed by any subsequent events. The removal of younger species from the "Mio-Pliocene" tree minimized most of the possible effects of the subsequent radiation during this period which could obscure older relationships.

RESULTS AND DISCUSSION

Analysis 1 ("Total" tree). Four equally most parsimonious trees, with length 703, CI = 0.21, and RI = 0.52, were obtained. The main differences in tree topology were related to the relative positions of taxa 17 and 18. In one case taxon 17 was hypothesized to be sister of the group formed by taxa 10 and 18, whereas in the other case taxon 18 was hypothesized to be sister of the group formed by taxa 10 and 17. The former tree was accepted because it was more consistent with the observed fossil record of the group. Fig. 1 shows a reconstruction of the accepted tree plotted against the stratigraphic column (the "Total" tree).

Fig. 1 shows striking stratigraphic inconsistency. One-third of the branches are ghost lineages that extend for more than ten million years without a single fossil occurrence or with fossils that appear several million years after their origination times as hypothesized by the cladogram (*i. e.* taxa 9, 12, 40/41). The tree also hypothesizes that several extant species are ancestors of species that became extinct 10-15 Ma. This is the case for taxon 12, a living species reported from a region that yielded many other fossil species, but of which there are no fossil occurrences. According to the relationships postulated in Fig. 1, this

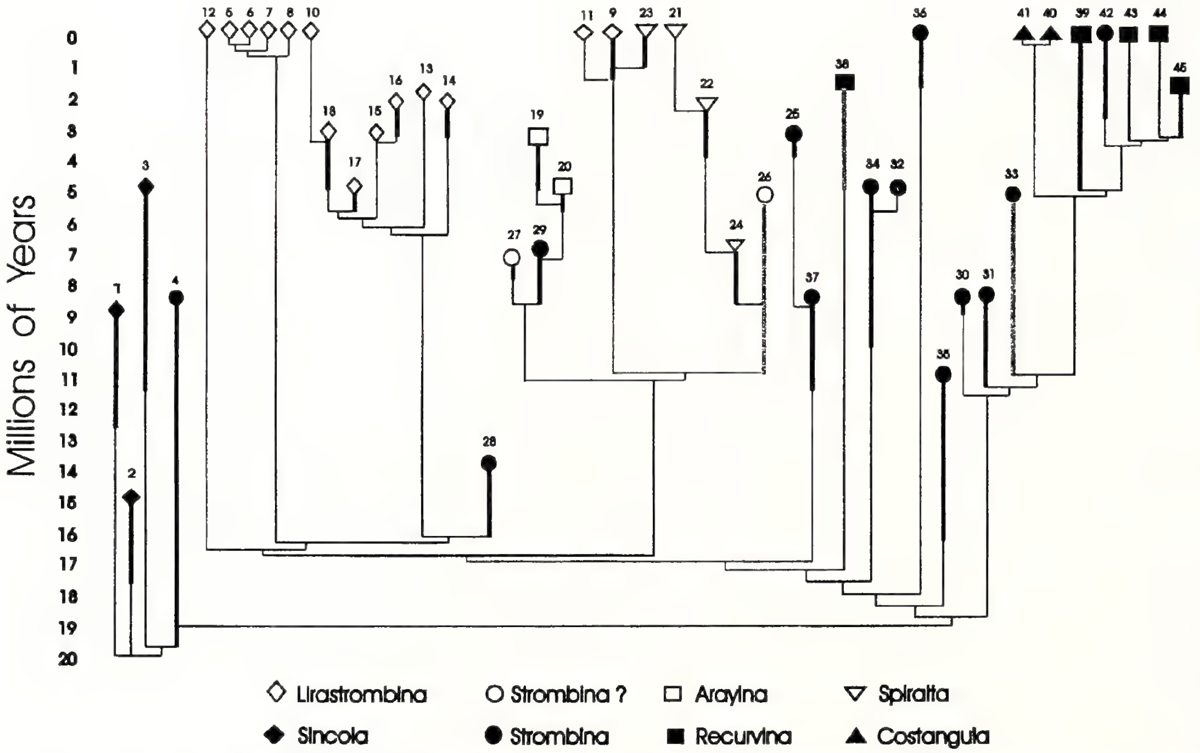


Fig. 1. Reconstructed 42-species cladogram ("Total" tree) showing the geologic time scale for calibration. Numbers on terminal branches represent species; symbols on terminal branches represent subgenera; thick black lines are real fossil data through time; gray lines represent ranges with uncertain age assignment; thin vertical lines represent ghost lineages hypothesized by the cladogram.

taxon is the ancestor of clades 13/18 and 5/8, as well as of taxon 28; however, most of these hypothesized descendent species became extinct several million years before taxon 12 originated. The same situation occurs with several other taxa (*i. e.* taxa 36, 38, etc.). These long ghost lineages also push back the radiation of the whole group to a time, approximately 15 Ma, when, although there is an excellent fossil record for many other molluscan groups in the same region, there is no sign of such a radiation in the *Strombina*-group.

Despite these stratigraphic inconsistencies, the subgeneric assignments of the species are mostly conserved, and several major clades are delineated, thus the problem is mainly one of stratigraphic inconsistency between clades and subclades. Such stratigraphic inconsistency is probably due to an extensive morphologic convergence caused by the evolution of similar forms during the turnover as existed in the earlier history of the group. Similar problems of homoplasy are common among corals, bryozoans, etc. (Budd and Coates, 1992; Jackson and Cheetham, 1994; Cheetham and Jackson, 1995, 1996).

Analysis 2 ("Mio-Pliocene" tree). This analysis yielded eight most-parsimonious trees, of 419 steps, CI = 0.31, and RI = 0.48. The major differences among these trees were in the basal taxa. In several trees the node that joined taxa 30 and 31 was basal to the rest of the tree, whereas in other trees the node joining taxa 28 and 35 was basal. The tree in which taxa 28 and 35 were basal was accepted because these taxa have older origination times.

The "Mio-Pliocene" accepted tree is plotted stratigraphically in Fig. 2. Most of the long ghost lineages have disappeared. None of the younger species included in the analysis are hypothesized to be ancestors of much older taxa. Once again, the subgeneric taxonomic assignments hold together and two major clades are clearly defined.

Analysis 3A (18 species, *"Strombina/Recurvina"* clade). The right subclade of the "Mio-Pliocene" tree (Fig. 2) comprised all species of the subgenus *Recurvina* Jung, 1989 (closed squares), and nearly all species of *Strombina s. s.* (closed circles). Three *Strombina s. s.* species (taxa 25, 26, 27) grouped with the left clade. This result supports the initial questionable subgeneric assignments of taxa 26 and 27 (Jung, 1989) and suggests that taxon 25 could also belong to another subgenus. The analysis of the 11 Mio-Pliocene species of these subgenera, plus the oldest *Strombina s. s.* (taxon 4) (which consistently appeared in a basal position in all previous analyses) rooted on *Sincola,* yielded only one tree with exactly the same topology, thus confirming the stability of this clade.

When the two younger species of *Strombina*, three of *Recurvina*, and two of *Costangula* Jung, 1989 (which had consistently grouped with the other two subgenera in

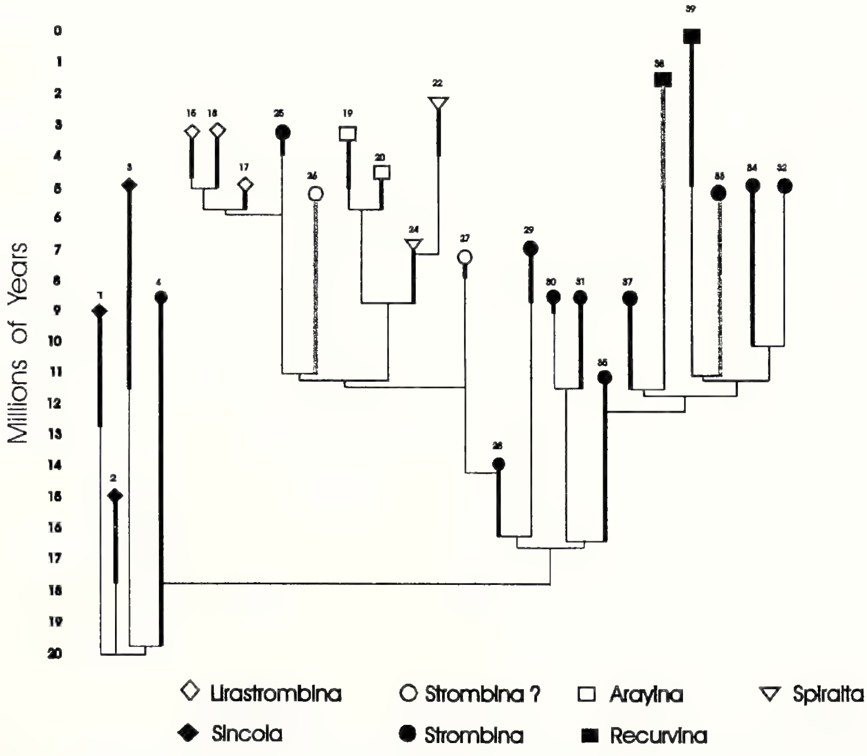


Fig. 2. Reconstructed 22-species cladogram ("Mio-Pliocene" tree) with geologic time scale for calibration. All symbols as in Fig. 1. This tree was the fixed point for Analyses 3A and 3B.

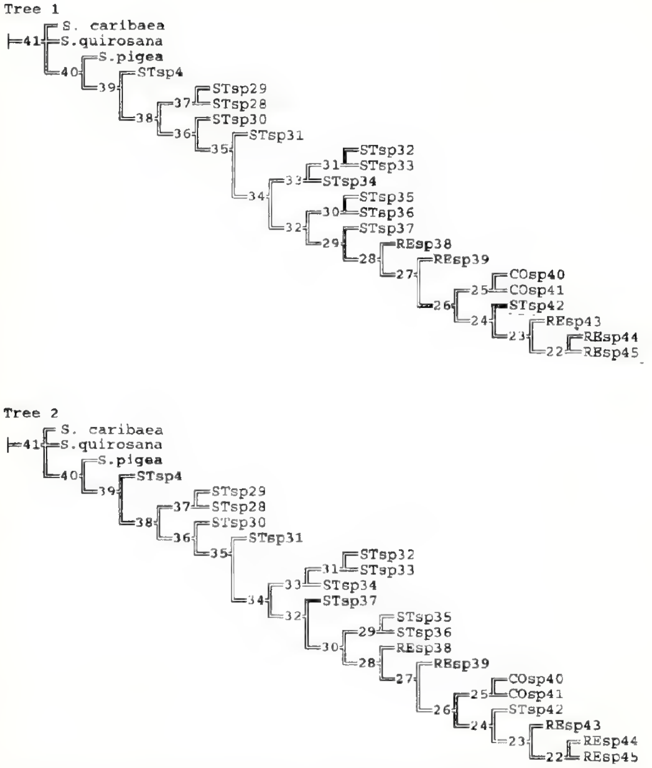


Fig. 3. Two most parsimonious trees yielded by Analysis 3A, including 19 species: 11 Mio-Pliocene species of the subgenera *Strombina* (ST) and *Recurvina* (RE) (= right clade of the "Mio-Pliocene" tree) plus seven extant species: two *Strombina s. s.*, three *Recurvina*, and two *Costangula* (CO) rooted on three species of *Sincola* (*S. caribaea* Gabb, 1873; *S. quirosana* H. K. Hodson,1931; *S. pigea* Olsson, 1964). The oldest *Strombina s. s.* in the analysis (taxon 4) was also used here.

the "Total tree" analysis), were added to the analysis and rooted on *Sincola*, the resulting two most-parsimonious trees (Fig. 3) differed only in the relative position of taxon 37 versus 35 and 36. The tree in which taxon 35, the oldest, branched first (Fig. 3, Tree 1) was accepted. Fig. 4 shows the reconstructed "Mio-Pliocene" tree (Fig. 2) with the *"Strombina/Recurvina"* clade (Fig. 3) added to the right side.

Analysis 3B (23 species, *"Lirastrombina/Spiralta/Arayina"* clade). The left clade of the "Mio-Pliocene" tree comprised all species of three subgenera: *Lirastrombina* Jung, 1989 (open diamonds), *Spiralta* Jung, 1989 (open inverted triangles), and *Arayina* Jung, 1989 (open squares), as well as the three *Strombina s. s.* (taxa 25/27) mentioned above. Analysis of the ten Mio-Pliocene species of these subgenera plus the oldest *Strombina* species (which had been basal in all prior analyses), rooted on *Sincola*, yielded five trees. The major differences among these trees were in the relative positions of taxa 25 and 26. The tree in which the node that joined taxa 25 and 26 was basal was accepted based on the older origination times of taxon 26. This topology also retained taxa 26 and 27 close together. In spite of these changes, all topologies maintained all species belonging to the left clade in the same relative positions they had in the "Mio-Pliocene" tree, thus confirming the stability of this clade.

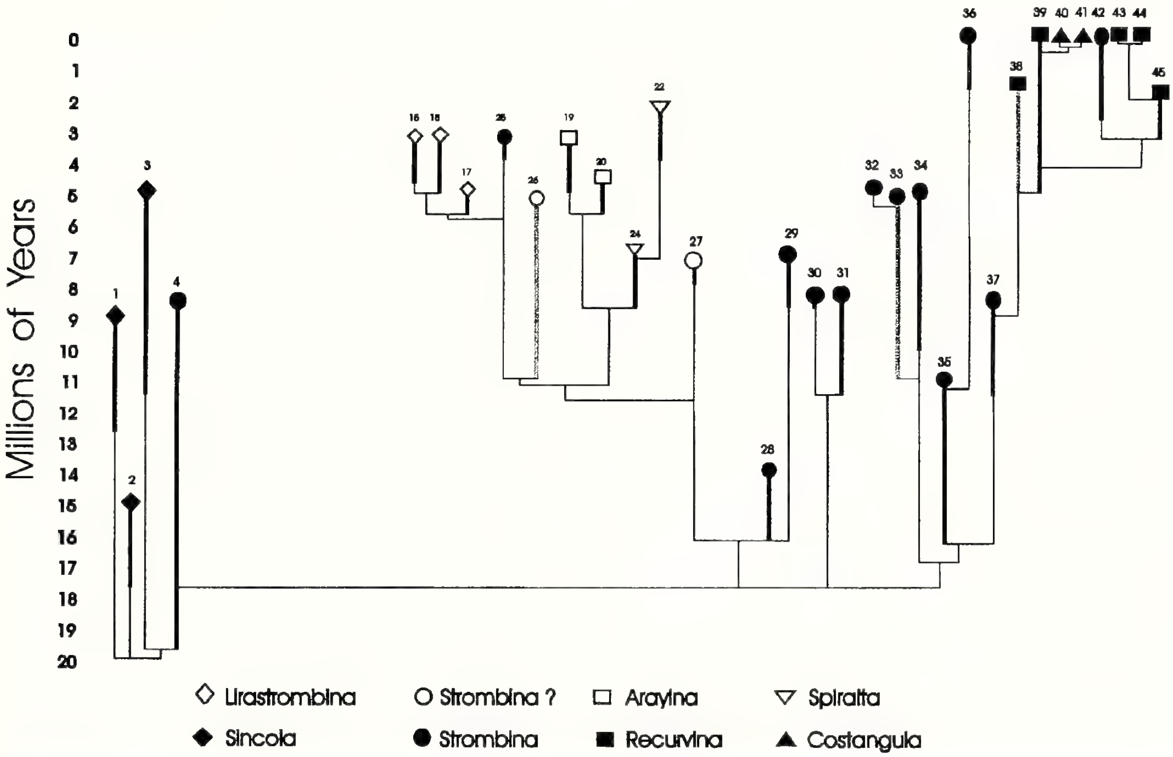
The *"Lirastrombina/Arayina/Spiralta"* clade involved three different and large subgenera, and it was analyzed in two separated phases. In phase 1, when the ten extant species of *Lirastrombina* were added to the three Mio-Pliocene species plus the *Strombina s. s.* (taxon 4) and rooted on *Sincola*, the resulting two most-parsimonious trees (Fig. 5) differed only in the relative positions of taxa 13 and 14. In one of these trees, taxon 14 was hypothesized as a sister taxon of taxon 13, whereas in the second tree, taxon 13 was hypothesized as branching earlier. Because taxon 14 has an earlier origination time than taxon 13, the tree in which it is hypothesized to arise at least at the same time (as a sister taxon of 13 and not later) was accepted (Fig. 5, Tree 1). Due to extreme homoplasy, the extant taxon 10 always nested very deep within the *Lirastrombina* tree, resulting in a decrease of overall stratigraphic consistency. This problem was solved by the elimination of this species from this phase of the analysis; this species was later added to the "Total tree" based on its position in the first analysis (see Fig. 1).

In phase 2, when the two extant species of *Spiralta* were added to the Mio-Pliocene species of *Spiralta* and *Arayina* plus taxon 4 and rooted on *Sincola*, the result was a single tree (Fig. 6).

Analysis 4 (composite tree). The trees resulting from Analyses 2 and 3 were then added to the left side of the "Mio-Pliocene" tree. Fig. 7 shows the final reconstructed tree. This tree has a high stratigraphic consistency even though ages were not used directly as characters. It has two major clades: one composed of members of the *Strombina, Recurvina,* and *Costangula* subgenera; the other is composed mainly of members of the *Lirastrombina, Arayina*, and *Spiralta* subgenera. These same major clades were delineated in the original 42-species tree (Fig. 1), but some of their relationships were originally obscured by morphologic convergence which then confused the stratigraphic relationships. A few ghost lineages, presumably due to missing fossil data, still remain.

CONCLUSIONS

The observed stratigraphic order of species occurrences (first and last appearances) should be used as one more source of data in phylogenetic inferences. The technique proposed here is a simple and independent way of assessing rival phylogenetic hypotheses obtained through parsimony methods. The final tree is more consistent with


Millions of Years
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
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18
19
20
1
2
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4
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18
17
25
26
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22
24
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
◇ Lirastrombina
◆ Sincola
O Strombina ?
● Strombina
□ Arayina
■ Recurvina
▽ Spiralta
▲ Costangula

Fig. 4. Reconstructed cladogram of the 22 Mio-Pliocene species plus the seven extant species of the right clade (*"Strombina/Recurvina"* clade), with geologic time scale for calibration. All symbols as in Fig. 1.

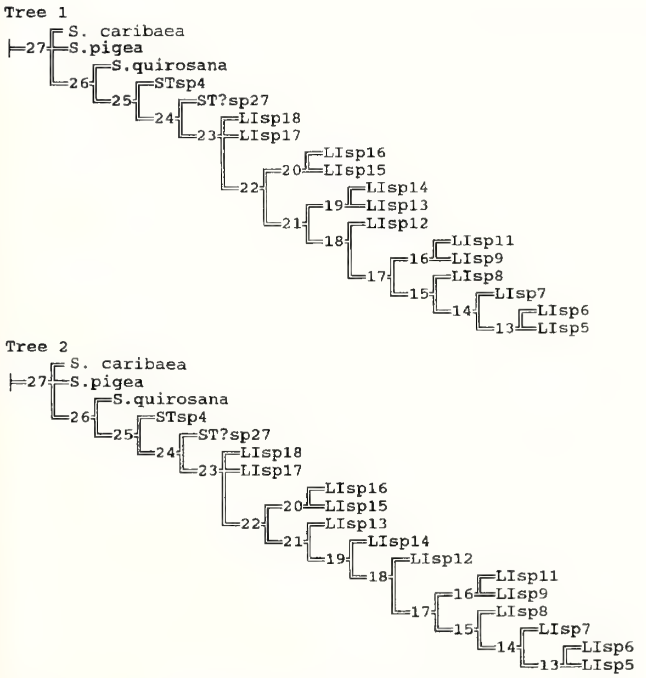
Tree 1
S. caribaea
27
S.pigea
S.quirosana
26
STsp4
25
ST?sp27
24
LIsp18
23
LIsp17
LIsp16
20
LIsp15
22
LIsp14
19
LIsp13
21
LIsp12
18
LIsp11
16
LIsp9
17
LIsp8
15
LIsp7
14
LIsp6
13
LIsp5
Tree 2
S. caribaea
27
S.pigea
S.quirosana
26
STsp4
25
ST?sp27
24
LIsp18
23
LIsp17
LIsp16
20
LIsp15
22
LIsp13
21
LIsp14
19
LIsp12
18
LIsp11
16
LIsp9
17
LIsp8
15
LIsp7
14
LIsp6
13
LIsp5

Fig. 5. Two most-parsimonious trees yielded by the analysis of 13 species (Mio-Pliocene plus extant) of the subgenus *Lirastrombina* (LI) rooted on *Sincola*. This was the first phase of Analysis 3B. Other taxon labels as in Fig. 3.

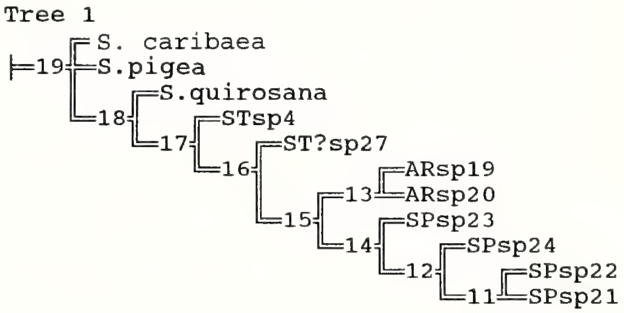
Tree 1
S. caribaea
19
S.pigea
S.quirosana
18
STsp4
17
ST?sp27
16
ARsp19
13
ARsp20
15
SPsp23
14
SPsp24
12
SPsp22
11
SPsp21

Fig. 6. Single most-parsimonious tree yielded by the analysis of eight species (Mio-Pliocene plus extant) of the subgenera *Spiralta* (SP) and *Arayina* (AR) rooted on *Sincola*. This was the second phase Analysis 3B. Other taxon labels as in Fig. 3.

the fossil data and uses fewer statements about relative probabilities of preservation, thus conforming to the principle of parsimony.

Several main aspects of the methodology used and its implications for the study of phylogenetic relationships should be noted here. Others will be considered in a later work dealing mainly with further refinement of this technique, as well as with its comparison with similar methods. The quality of geochronological data used is very important for the correct interpretation of the results obtained with

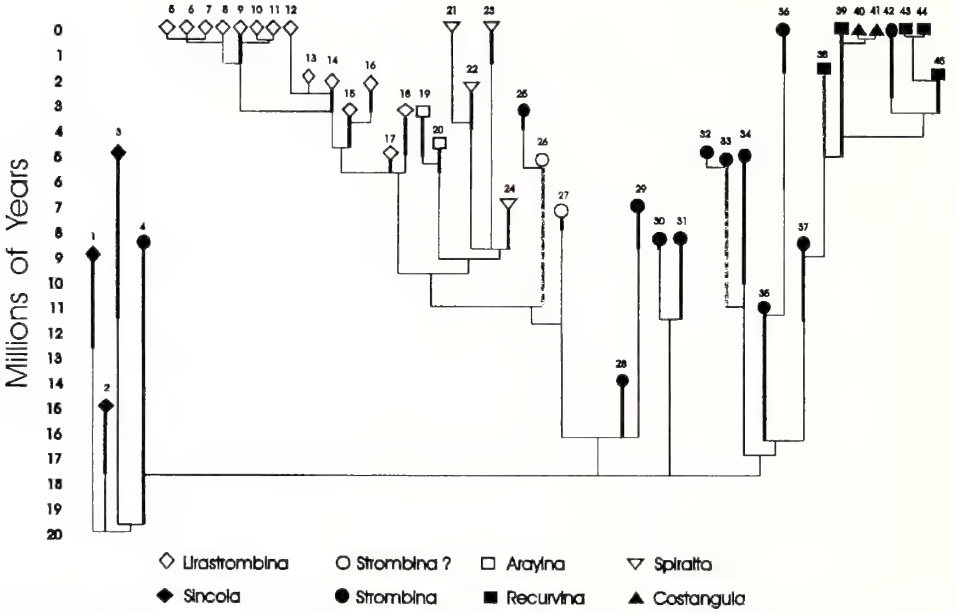

Fig. 7. Reconstructed cladogram including the 42 initial species. This is the final composite tree based on the "Mio-Pliocene" tree plus the trees yielded by Analyses 3A and 3B. All symbols as in Fig. 1.

this methodology. Uncertain age assignments and low stratigraphic resolution will make difficult the decision about time intervals into which to divide the analyses, thus increasing the uncertainty in recovering the true history of the clades under study. Sampling can also be a problem: uneven geographic and stratigraphic sampling will give an incomplete picture of the true abundance and diversity of the groups studied, and its effects can be confounded with homoplasy. Another important point is the question of how well the species-level taxonomy is employed. The "Total tree" preserved most of the subgeneric units (with only a few exceptions, some of which were already doubtful). This result matches earlier work (Fortunato and Jung, 1995) using both shell morphology and anatomy, thus adding confidence to the methodology employed to recover the true history of the clade. In spite of this agreement with the structural component of the analysis, the temporal aspect was violated both by the extremely long-lasting ghost lineages and by the hypothesized order of originations of several ancestor-descendant pairs. The removal of all taxa that originated during the last three million years resolved many of these incongruences with the known order of fossil occurrences. Most of the hypothesized cladistic relationships (*i. e.* ancestor-descendant pairs and sister taxa) among the Mio-Pliocene taxa have a high level of agreement with their origination time as shown by the fossil record. This also helped to resolve most of the long-lasting ghost lineages. These results all agree with the assumption that most of the stratigraphic inconsistencies present in the first tree (Fig. 1) were due to high levels of homoplasy after and before the turnover event rather than to the incompleteness of the fossil record, incorrect taxonomic assignments, or sampling bias.

It is important to notice that the evolutionary patterns hypothesized by the trees obtained before and after the removal of the young species are very different. These evolutionary scenarios and its implications for the history of the group will be analyzed elsewhere. It is enough to mention here that whereas the tree represented in Fig. 1 implies an extensive radiation of the whole group approximately 15 Ma, that represented in Fig. 6 suggests that there were two radiations (or three), one approximately 10 Ma, and the second probably just before the final closure of the isthmus of Panama, and extending for some time thereafter in the eastern Pacific. This scenario of several possible radiations agrees with the fossil record.

The technique outlined in this paper is still in its preliminary stage. As such it suffers from several biases which are currently being resolved. One of these is the lack of stratigraphic confidence intervals for the samples. The approach used here assumes that the sampling bias is not large enough to significantly override the effects of homoplasy. As part of the development of this technique, stratigraphic intervals will be calculated following Wagner's (1995) methodology. This will separate the effects of homoplasy from those of inadequate sampling, thus testing the consistency of the method. Also as part of the work in progress, the same data set will be employed using Fisher's methodology (Fisher, 1992; Clyde and Fisher, 1997) and the resulting trees compared with the composite tree obtained through the technique presented here. This will provide a test of the power of both methodologies and evaluate their capability of recovering the true history of this group.

Regardless of the need for further development of this technique, it is clear that conventional parsimony analyses are only the first step towards recovering the true history of a clade. Cladistic hypotheses should be tested against the stratigraphic data if at all possible. Failure to do this can distort the evolutionary histories and falsify our predictions about evolutionary patterns.

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Review of shell reduction and loss in traditional and phylogenetic molluscan systematics, with experimental manipulation of a negative gain character

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Abstract: Modern phylogenetic methods are improving our basis for molluscan systematics and our understanding of evolutionary processes. The use of traditional characters in a phylogenetic analysis helps us directly contrast their accustomed "taxonomic value" with synapomorphies suggested by a cladogram. While most cladistic characters are structurally complex, opisthobranch and pulmonate gastropods exhibit numerous characters which are losses - in shell, operculum, radula, etc. - some as presumed synapomorphies for higher-level taxa. These losses, called negative gains, can be complete (absence) or partial (reduction). To describe and code such characters, we are forced to assess morphology that is not observable. How we do so can affect tree topology, and thus the final hypothesis. This in turn determines what sequences of character evolution are supported, what monophyletic clades are recognized, and if translated into a hierarchical classification, what established taxa are confirmed or rejected. Negative gain characters must be explicitly defined to ensure repeatability; in particular, "reduced" must consider the type of reduction (in size or composition), the level of reduction (expressed qualitatively or numerically), and presumed homologies of an unobservable feature. Inclusion of negative gain characters in an analysis can document the extent of putative parallelism or "trends" for character loss in a lineage; still, subjective coding decisions have profound effects. These points are illustrated here by three datasets derived from recent literature, on sacoglossan and notaspidean opisthobranchs and on sigmurethran pulmonates, in which the shell exists in fully present, reduced, and fully absent states. By manipulating only shell characters in these multi-system datasets, through different *a priori* assumptions and coding alternatives (binary, multistate unordered, multistate ordered, uncoded/mapped), changes in the resulting cladogram(s) were induced, ranging from extreme, to slight, to unchanged. In the absence of methodological preference, the use of multiple methods is advised, with conclusions based on all results and on confidence in carefully coded characters.

Key words: cladistics, classification, Mollusca, Opisthobranchia, Pulmonata

In the last decade of systematic malacology, phylogenetic methodology (= cladistics) has allowed the re-evaluation of traditional molluscan systematics in ways that are more objective and repeatable than previously possible. Although the advantages of this technique are now well-accepted, cladistic analyses often suggest dramatically different evolutionary relationships, and thus hierarchical taxonomic classifications, than those long-considered as dogma. Because ranked classifications are necessary means of convenience and communication, radical changes from familiar taxonomic arrangements can place the phylogeneticist at odds with workers in applied systematics (*e. g.* education, ecology, collection management, biopolitics) as well as other disciplines (*e. g.* neurophysiology), needing proper labels for their research subjects. The systematic research community is likewise impacted when newly-named taxa, created in a fervor to label clades, are rapidly overturned, often by the same author(s) (*e. g.* Triganglionata Haszprunar, 1985b = Allogastropoda Haszprunar, 1985a). It is therefore prudent to be confident of cladistic results before traditional arrangements are summarily discarded in favor of phylogenetic ones.

Whenever cladistic revision of a taxonomic group is attempted, two questions must be addressed: (1) are the traditional taxa monophyletic clades? and (2) are the traditional taxon-defining characters synapomorphies? The most effective method of answering both of these questions is to actually code and use traditional characters as part of the phylogenetic dataset. Only by doing so can the accustomed "taxonomic value" of a character be directly compared with the pattern of character evolution revealed by a cladogram.

Cladistics depends on the inheritance of derived characters, and performs optimally when these characters are structurally complex. But in many traditional characters of many taxa, the derived condition is absence (= presumed secondary loss of a primitively present feature) and is called a negative gain. In cladistic terms, instead of plesiomorphic 0 = absent, and derived 1 = present, one has the reverse: 0 = present, 1 = absent. Difficulties are compounded when an intermediate stage is involved, *i. e.* when the feature is still present but appears reduced or simplified in some fashion. The intermediate state changes the character from discrete (present/absent) to continuous (coded as 0 = present, 1 = reduced, 2 = absent), wherein the character state boundaries (especially between present and reduced) can be ambiguous.

Traditional systematics is replete with examples of taxa defined (at least in part) by derived absences, *e. g.* reptiles without limbs (Serpentes; snakes). Among mollusks, Ponder and Lindberg (1997: 205) suggested that "loss of plesiomorphic structures, rather than their structural modification, accounts for much of the homoplasy seen in gastropods." Examples of traditional taxonomic losses across the phylum include the entire jaw/radula complex in Bivalvia, ctenidia in Scaphopoda and Heterobranchia, the operculum in Marginellidae and most Olividae, the radula in Pyramidellidae and Retusidae, and jaws in Neogastropoda (see Boss, 1982; Willan, 1987; South, 1992; Salvini-Plawen and Steiner, 1996). Nearly half of the traditional taxonomic characters of Cephalaspidea (Opisthobranchia; "bubble snails") involve reduction or loss (= total reduction) of a feature (Mikkelsen, 1993). Reduction of the shell is probably the most-cited example, and has presumably occurred many times throughout the course of molluscan evolution. Reasons proposed for such losses include: ecophenotypic (streamlining for burrowing, flexibility for slithering into crevices), physiological (scarcity of calcium in the environment, constraints of parasitic life), and developmental (miniaturization, paedomorphosis). [Fong *et al.* (1995: 251) suggested that loss might have less to do with adaptation than with "indirect selection" when there is "relaxation of ... stabilizing selection" on a character; they viewed the reductive process as evolutionarily polarized, from nonfunctionality, through atrophication, to complete loss.] Regardless of the actual underlying cause, when shell reduction is considered "an evolutionary trend" or characteristic of a taxon, it becomes a negative gain character in cladistics.

Although negative gain characters are not impossible to accommodate in cladistics, they can be "especially problematic" (Bieler, 1992: 315; Mikkelsen, 1993). First, because homology centers on structure for recognizing homologous states of a character (by at least one definition; see reviews by Hall, 1994), negative gains are more difficult to code because there is nothing (in the case of absent features) to interpret. Second, because the term "reduced" can encompass a broad range of factors (reduced in size, thickness, sculpture, complexity, function, etc.; Pogue and Mickevich, 1990; Proctor, 1996), one must assure that the reduced state of one taxon is the same (homologous?) with that of the other taxa being investigated; thus precise definition of kind and amount of reduction is required. Third, how we code absence is controversial: although unknown or inapplicable character states are most often treated as question marks in datasets, Pimentel and Riggins (1987) advocated that absence is a valid character state when it is apomorphic. Finally, because reduction or loss of a feature can conceivably occur more than once in a lineage, negative gains carry the threat of increased homoplasy (= extra steps in an analysis, reflected in a character consistency index of < 1.0). So, negative gain characters raise recurrent uncertainties about: (1) homology (is the present feature homologous with the absent feature?), (2) definition (how do we describe something we can't see?), (3) procedure (how do we code absence and/or reduction?), and (4) relative usefulness (should we omit such characters from the analysis if they are inherently homoplastic?).

Focussing on the molluscan shell, the goals of this study are: (1) to review shell reduction and loss as an example of a traditional qualitative character in mollusks; (2) to discuss how shell reduction and loss can be used as a negative gain character for cladistic analysis, in terms of implied homologies, requisite definition, and inherent difficulties; (3) using published datasets, to show how results and conclusions can change with experimental manipulation of shell reduction and loss characters; (4) to consider the pros and cons of coding and analytical alternatives; and (5) to emphasize the cladistic utility of negative gain characters, as means of hypothesizing the occurrence of homoplasy and testing accustomed taxonomic value.

DEFINITION AND REVIEW OF SHELL REDUCTION

A large external shell composed of calcium carbonate and secreted by the mantle is synapomorphic for, and thus plesiomorphic within, conchiferan mollusks (Brusca and Brusca, 1990; Lindberg and Ponder, 1996). Absence (= loss) of the shell is therefore apomorphic within the group (Ponder and Lindberg, 1997). Ontogenetic evidence supports this interpretation; a shell is present in the larval form of most shell-less mollusks. Loss of the adult shell has presumably occurred in parallel in octopod Cephalopoda and a number of times in two major gastropod lineages: Opisthobranchia (including some Anaspidea, Notaspidea, and Sacoglossa, and all Nudibranchia and Gymnosomata; see Gosliner and Ghiselin, 1984; Gosliner, 1991), and Pulmonata (including especially Soleolifera and Philomycidae; see South, 1992). Opisthobranchs are most renowned in this regard; as noted by Solem (1974: 117), "The one clear trend in evolution [of opisthobranchs] is toward loss of the shell."

Several lineages of mollusks show "the most clearly defined trend in shell variation" (Solem, 1974: 16) where the derived loss is less than complete, *i. e.* where the shell is present but reduced. A reduced (= vestigial, rudimentary) shell is listed as a traditional character for higher taxa from the subclass (*e. g.* Opisthobranchia) to family (*e. g.* Teredinidae) level (Table 1). However, shell reduction can encompass: (1) reduced in size relative to the overall size of the body (also generally meaning that the mollusk cannot fully retract into its shell), and/or (2) reduced in thickness, *i. e.* thin-walled, fragile, and/or weakly calcified. While not qualifying as shell reduction alone, other recurring attributes of reduced shells include: (1) auriculiform or plate-like shape, with an oversized body whorl (= rapidly expanding whorls), and enlarged aperture; (2) streamlined with regard to reduction in spines, ribs, and other sculpture; and (3) completely internal or incompletely internalized by hypertrophied, overlying mantle folds.

Evolutionary modification from shell present to reduced, then, can represent different evolutionary pathways, and caution must be used that one is coding only one kind of reduction within a single transformation series. For example, the large, paper-thin shell of a *Haminoea* (Opisthobranchia: Cephalaspidea: Haminoeidae) and the small, thick one of a *Fissurellidea* (Vetigastropoda: Fissurellidae) can both be called reduced, therefore theoretically they could both be identically coded. Yet the character state change is not identical - one is reduced by becoming thinner, the other by becoming smaller. In most taxa, the shell is actually reduced in both size and thickness (Table 1). Thus the transformation is a mixture of two different evolutionary pathways that can occur together or independently. Although the shell itself is homologous within Mollusca, the reduced state of one shell might not be homologous with the reduced state of another. Likewise the absent state of one shell (*e. g. Bursatella*, an anaspid) is not necessarily homologous with the absent state of another (*e. g.* a nudibranch). Proctor (1996: 144) recognized this problem by stating that "losses of a character state may be falsely homologized, since although there may be many independent losses of a character state, seldom are there structural or behavioral clues to this independence." In-depth examination of molluscan larval shells, which are present in nearly all shell-less groups (Thompson, 1976), could provide ontogenetic evidence for decision-making in this area.

Table 1. Supraspecific taxa listing "shell reduced" [but not meaning "absent"] as a traditional character. Based on descriptions by Boss (1982) unless otherwise noted. (S, reduced in size; T, reduced in thickness).

"Prosobranchs"	
Fissurellidae: *Fissurellidea* group (McLean, 1984)	S
Naticidae: Sininae	ST
Lamellariidae	ST
Carinariidae	ST
Opisthobranchs	
Cephalaspidea	ST or T
Runcinoidea	ST
Philinoglossacea	ST
Sacoglossa: Oxynoidae	ST
Anaspidea: Notarchidae	ST
Notaspidea	S
Pulmonates	
Amphibulimidae	ST
Limacidae	S
Testacellidae	S
Bivalves	
Galeommatoidea	ST or T
Teredinidae	S
Cephalopods	
Coleoidea (also Brusca and Brusca, 1990)	ST
Teuthoidea (also Brusca and Brusca, 1990)	ST
Loliginidae	ST
Octopoda (also Brusca and Brusca, 1990)	ST
Cirrata	ST
Opisthoteuthidae	ST
Incirrata	ST
Boltaenidae	ST

In addition to defining the pathway of reduction, the limits of reductive character states must be clearly defined. How much smaller or thinner does the shell have to be, to be coded as reduced rather than as fully present? Will levels of reduction be coded as separate character states, and if so, are these meaningful levels, or arbitrary cut-offs within a continuous morphocline? Several solutions have been utilized in the past: capacity of the animal to withdraw into the shell (Boss, 1982); and relative sizes of the shell and mantle, expressed qualitatively or as a numerical ratio (McLean, 1984; Willan, 1987; Bieler and Mikkelsen, 1992).

In summary, for coding and analyses to be repeatable, defining the shell condition (*i. e.* the kind and degree of reduction) is a fundamental step. To minimize *a priori* reasoning, shell condition must be as explicitly defined as possible, ideally without the use of imprecise terms such as vestigial or rudimentary.

CODING ALTERNATIVES AND METHODS

In this study, published datasets were used to illustrate if and how a change in coding adult shell reduction can produce different results. For these purposes, I am assuming that reduction has been in each case rigorously defined as required above, limiting the reduced state to one homologous pathway and one level. In each case dataset, four alternative coding choices are used, reflecting different *a priori* assumptions and phylogenetic philosophies.

Binary Coding (two separate binary characters: (1) 0 = present, 1 = absent; and (2) 0 = fully present, 1 = reduced): In this method, taxa with shells absent (character 1 = 1) are coded "?" (= unknown or inapplicable) for character 2 because the appearance of something that is not present cannot be determined (Maddison, 1993). [This is not the same as additive binary coding, which divides a multistate character into subcharacters and produces the same cladogram as additive (= ordered) multistate character (Hauser and Presch, 1991; Wiley *et al.*, 1991).]

Multistate Unordered Coding (single multistate character: 0 = fully present, 1 = reduced, 2 = fully absent): This coding avoids the "?"s necessary with Binary Coding (above). All characters were treated as fully unordered (= non-additive, minimally connected), that is, considering any character state change (0 to 1, 1 to 2, 0 to 2, plus reversals) as possible in a single step.

Multistate Ordered Coding: Same coding as the previous, but this character (only) was treated as a linearly ordered character (= additive, maximally connected). This assumes that reduced is a requisite intermediate stage between fully present and fully absent, therefore, *e. g.* a change from 0 to 2 requires two steps instead of one. All other multistate characters in each dataset were left unordered.

Uncoded/Mapped: Here shell condition was not coded or used in the analysis, but was subsequently mapped onto the resultant tree(s). This method has been used by authors when data are missing in a large number of taxa (Mikkelsen, 1996), or when negative gain characters are thought to be overly homoplastic (Ponder and Lindberg, 1996, 1997; although one survey [Proctor, 1996] found that potential homoplasy has seldom been cited as a reason to exclude characters). This reflects a decision, *a priori,* not to allow the character to play a role in tree construction.

Each trial or test dataset, then, consisted of four analyses: Binary Coding, Multistate Unordered Coding, Multistate Ordered Coding, and Uncoded/Mapped. Each used the parsimony-based algorithms of Hennig86 (Farris, 1988), using in each trial an algorithm which resolved in a reasonable amount of time and yielded a total number of trees which could be rapidly analyzed (i. e. no memory overflows). Because this is a demonstration, it was not important that these be rigorous analyses, only comparable ones. The same algorithm was used within each trial set of four analyses; all characters were given equal weight (= unweighted) in all analyses. Uncoded/Mapped analyses used the binary datasets, but with shell-reduction characters inactivated within Hennig86. Character analysis was assisted using Clados (Nixon, 1992). Cladograms were rendered for publication using Component (Page, 1993).

Cladistic analyses most often result in more than one, often many, most-parsimonious trees (MPTs). Although "one would ideally examine the implications for character evolution on all equally acceptable phylogenies" (Maddison, 1991: 315), most authors condense the MPTs either through consensus trees or successive approximations weighting (Carpenter, 1988). Because these decrease the amount of information revealed by the analysis, an alternative method of summarizing topologies was used here. When a series of taxa occur in a consistent region of all trees, but in varying arrangements within that region, repetitive regional topologies can be identified that are much smaller in number than the total number of trees. Each regional topology can be examined separately for implied patterns of character evolution. For this discussion, only those regions in which character state changes relevant to shell reduction or loss occurred are presented in full. Other regions are abbreviated here, and the arrangement of taxa comprising each such region (although admittedly not unimportant to tree length and construction) was generally disregarded.

TEST DATASET RESULTS

Although manipulation of hypothetical datasets in studies such as these are often instructive, one is inevitably left wondering how similar manipulations would affect real data. Therefore, the early choice was made here to conduct these trials on actual datasets. Unfortunately, very few published datasets have used shell reduction and loss as coded characters, perhaps for the reasons cited above. Some studies have included a shell present/reduced character (*e. g.* Bieler and Mikkelsen [1992] coded 0 = subequal to mantle, 1 = significantly smaller than mantle; Jensen [1996a] used 0 = large, 1 = small), but to be most effective here, datasets using taxa with shells in at least three possible states (present/reduced/absent) were desired. Experimental results using three test datasets are here presented: sacoglossan (Jensen, 1996b) and notaspidean opisthobranchs (Willan, 1987), and sigmurethran pulmonates (Tillier, 1989). Each dataset included characters from a wide range of soft anatomy systems (*e. g.* mantle cavity, alimentary tract, nervous system, reproductive system) in addition to the shell characters. Summaries of the results in each section (below) emphasize: the number of MPTs, monophyletic clades supported (including traditional taxa, for ease of discussion), and interpretation of shell reduction/loss evolution from the cladogram(s). NOTE: Although these experimental trials used real datasets, the object was neither to revise the results of the original published version nor to criticize the original analysis. These matrices were analyzed here as experimental datasets for demonstration purposes only, and these results should not be interpreted as rigorous phylogenetic reanalyses of taxa. Support for named clades is mentioned only to illustrate changes in result depending on coding alternative used.

SACOGLOSSAN OPISTHOBRANCHS

The opisthobranch subgroup Sacoglossa (= Ascoglossa, "leaf-slugs") includes members with full-sized shells, others with shells reduced in size (both of these categories are reduced in thickness), and many without shells as adults. Shell presence/absence has played a traditional role in sacoglossan classification, with two groups, Oxynoacea and Placobranchacea, containing shelled and unshelled forms, respectively.

In 1996, Jensen published a phylogenetic analysis of the Sacoglossa, including 35 ingroup taxa (mostly at genus-level) and 52 characters from the shell, mantle cavity, gross morphology, circulatory, digestive, reproductive, and nervous systems, and egg mass. Her dataset (Jensen, 1996b: table 4) included five taxa with fully present shells, three with reduced shells, and 27 without shells as adults. Her character list treated the shell using three binary characters: (1) 0 = present, 1 = absent; (2) 0 = univalved, 1 = bivalved; and, (3) 0 = covering whole body, 1 = reduced. Character 2 accomodated shell condition in the famous "bivalved gastropods" (*Julia, Berthelinia*) and was not manipulated here. Characters 1 and 3 reflect shell loss or reduction in size, and were those involved in manipulation. Because Jensen's character list and data matrix (Jensen, 1996b: tables 3-4) were used here largely unchanged from the published version (altered only by adding an all-zero character 0 and correcting character 21 for *Mourgona* to 0; K. R. Jensen, pers. comm., 1996), they are not reproduced here.

The Hennig86 algorithms *mhennig** and *bb** (multiple passes plus branch-swapping) were used for these analyses. Several monophyletic clades were consistent and are abbreviated here for discussion: (1) 27 unshelled taxa hereafter combined as Placobranchacea; (2) the bivalved gastropods, *Julia* + *Berthelinia*, hereafter abbreviated as Juliidae; and (3) *Oxynoe* + *Lobiger* + *Roburnella* (usually united as a clade), hereafter (when monophyletic) as Oxynoidae. Variation within monophyletic Oxynoidae was disregarded, as was one taxon, *Cylindrobulla*, which was consistently basal and unresolved with the outgroup (Jensen's "Ancestor").

Binary Coding. Analysis of the original dataset produced 56 MPTs of length 155 (CI 0.41, retention index [RI] 0.76). These fell into eight topologies (Figs. 1-8: 1, 14 MPTs; 2, 12 MPTs; 3-5, eight MPTs each; 6-8, two MPTs each). Shell reduction (character 3 = 1) occurred independently of shell loss (character 1 = 1) (*i. e.* a branched character state tree) in six of the eight topologies (Figs. 3-8, 30 of 56 MPTs, or 54%). However, in spite of independent binary coding, shell reduction was prerequisite to shell loss (*i. e.* a linear character state tree) in the remaining two topologies (Figs. 1-2), which included the most frequently-occurring topologies (14 and 12 MPTs, respectively) and nearly half of the MPTs (26 of 56, or 46%). The traditional Oxynoacea was supported by four topologies (Figs. 5-8, 14 MPTs or 25%).

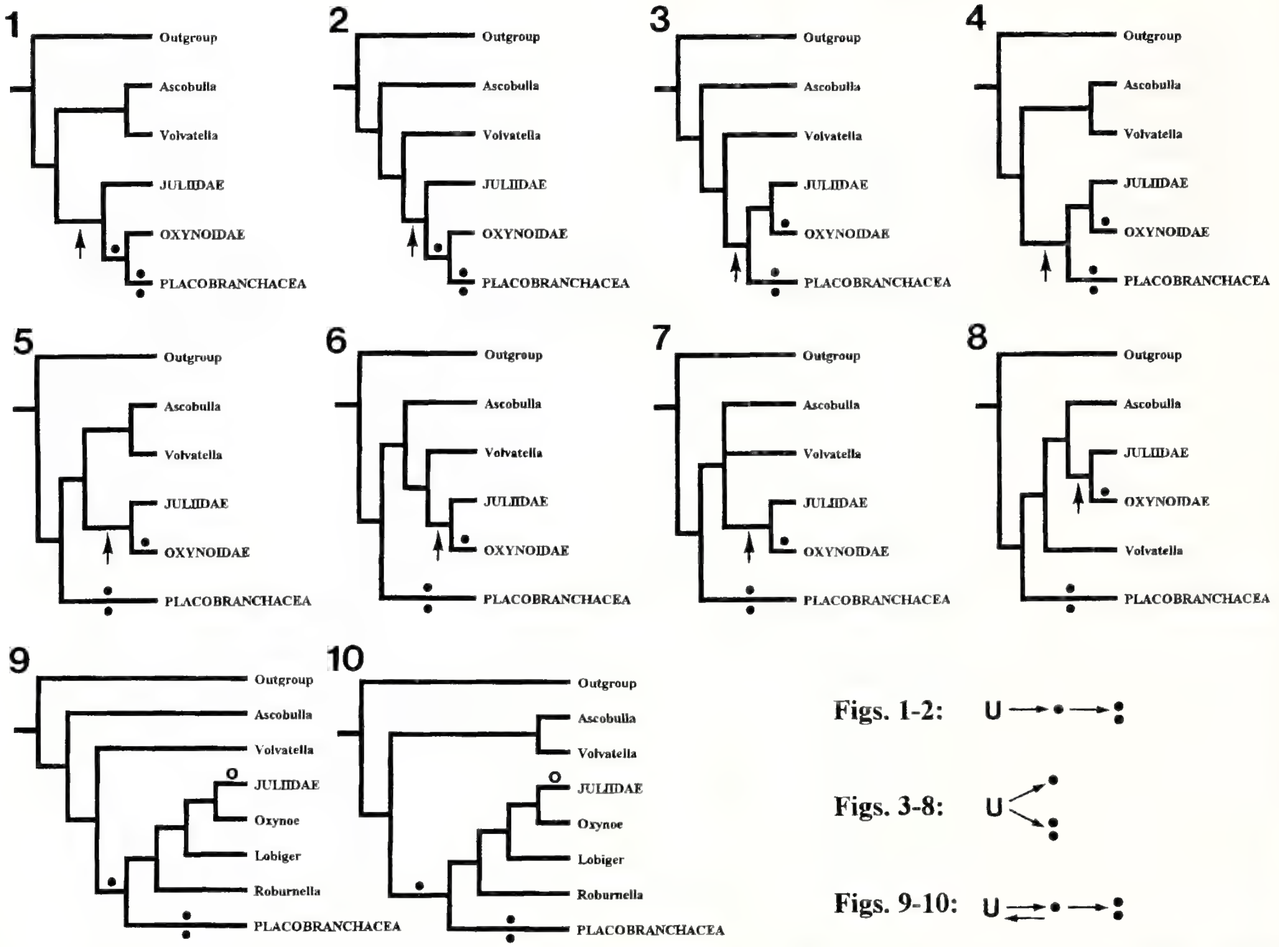
Multistate Unordered Coding. This method combined binary characters 1 and 3 to form a single multistate shell character: 0 = present, 1 = reduced, 2 = absent. The combined character replaced character 1, and character 3 was thus eliminated. The algorithm likewise produced 56 MPTs, in the same topologies as Binary Coding, and of nearly identical statistics (length 155, CI 0.41, RI 0.75). Character state trees were therefore also unchanged from Binary Coding.

Multistate Ordered Coding. Using the same dataset as Multistate Unordered Coding, this analysis was run with character 1 (only) ordered. 26 MPTs resulted, of length 155 (CI 0.41, RI 0.76). The result comprised only two topologies, identical in form (Figs. 1-2) and number (14 + 12 MPTs, respectively) to those requiring shell reduction prerequisite to loss in the previous two analyses (*i. e.* only those with linear character state trees). Unlike the previous two cases, traditional Oxynoacea was not supported by any of the resultant trees.

Uncoded/Mapped. This analysis used the original (binary) dataset, but with characters 1 and 3 inactivated. The result was 60 MPTs of 153 steps (CI 0.40, RI 0.75), including 56 trees of the same eight topologies realized by the previous analyses (Figs. 1, 14 MPTs; 2, 12 MPTs; 3-5, eight MPTs each; 6-8, two MPTs each), plus two new topologies (Figs. 9-10, two MPTs each). In these two new topologies (Figs. 9-10), the Oxynoidae became unresolved (apparently in the absence of its single synapomorphy, shell reduced [it was also united by two homoplastic character state changes in the nervous system]).

When mapped onto the trees, shell loss remained synapomorphic for the Placobranchacea, in evidence of support for the clade by three other synapomorphies (in vascular and reproductive characters) plus other homoplastic character state changes (in the nervous system). Shell reduction was independent of shell loss in 30 MPTs (Figs. 3-8, 50%); reduction was prerequisite to loss in the remaining 30 MPTs (50%), including the two new topologies (Figs. 9-10) and, again, the most frequently-occurring topologies (Figs. 1-2). Notably the two new topologies (Figs. 9-10) required reversals in shell reduction, from shell reduced back to fully present in Juliidae. Traditional Oxynoacea was supported by four topologies (Figs. 5-8, 14 MPTs or 23%).

Summary. (1) Change in coding from binary to multistate produced no change in the results if the analysis was run unordered. Ordering the shell reduction/loss character resulted in fewer MPTs, restricted to those which required shell reduction as an intermediate step before shell loss; in this case, this was a subset of the Binary or Multistate Unordered Coding results. Eliminating the shell reduction/loss characters produced more MPTs than any other coding alternative. (2) Regardless of coding alterna-

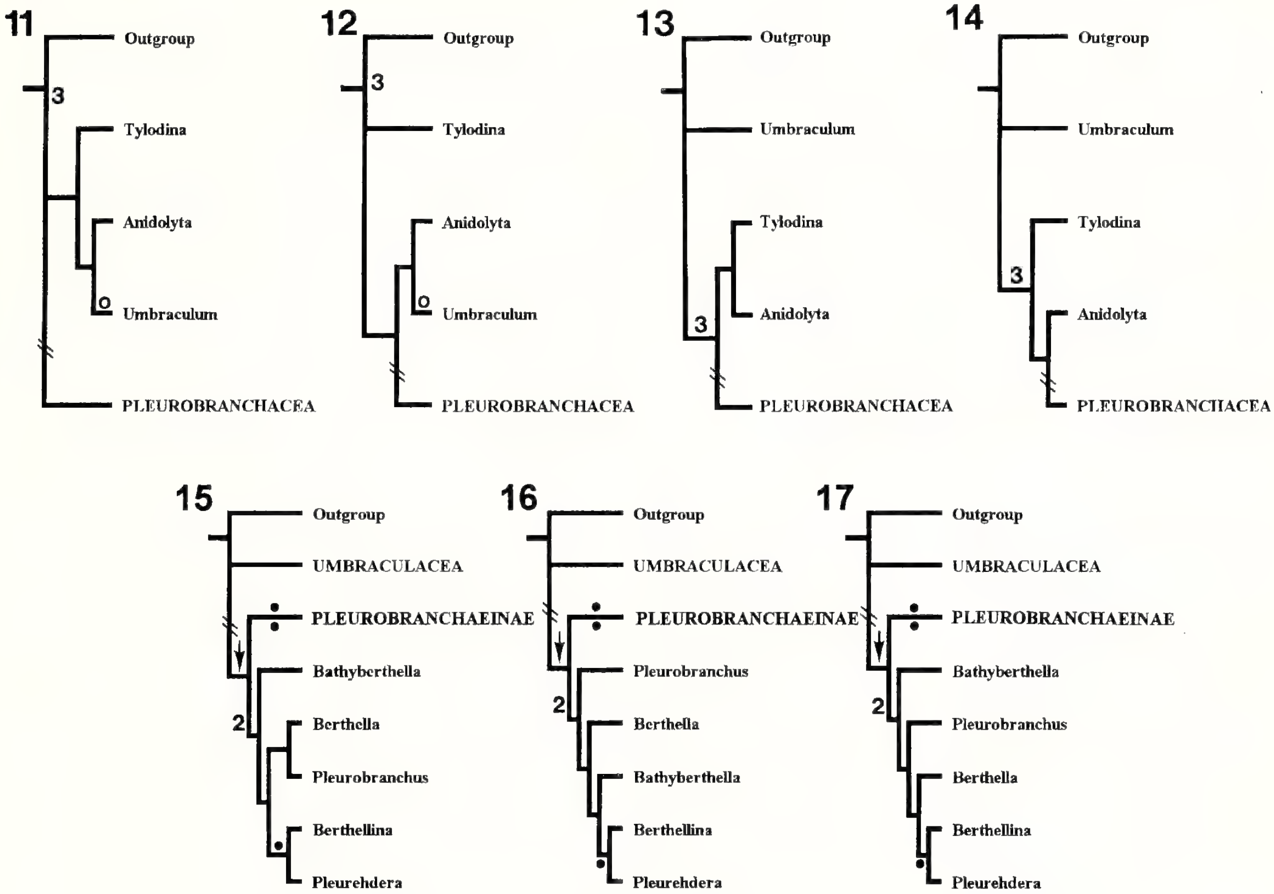


Figs. 1-10. Sacoglossan opisthobranch test dataset results and character state trees. Figs. 1-8. Most-parsimonious tree topologies from Binary, Multistate Unordered, and Multistate Ordered Coding. Figs. 9-10. Same, from Uncoded/Mapped results. (•, shell reduction; ••, shell loss; o, reversal to shell fully present; arrow, position of shell reduction if Juliidae shell is reduced before becoming bivalved [see text]).

tive, identical traditional taxa were generally supported. Placobranchacea consistently formed a clade even if shell reduction/loss was eliminated from tree construction. Oxynoacea was supported by most MPTs, but was unresolved in two topologies where shell reduction/loss characters were eliminated. (3) Regardless of coding alternative, shell reduction/loss characters usually served as synapomorphies for various clades: shell absent for Placobranchacea, and shell reduced for Oxynoidae (most cases). (4) Regardless of coding alternative, tree topology was generally sustained, even in Uncoded/Mapped analyses. (5) Shell reduction was prerequisite to shell loss in about 50% of the MPTs produced by each analysis, and in the most-frequently occurring topologies of each analysis. This was true even in unordered analyses. (4) Reversals occurred only in the Uncoded/Mapped analysis, involving only the Juliidae regaining a fully present shell from the reduced shell of Oxynoacea. This hypothesis could be envisioned if the bivalved gastropod shells of Juliidae could only be derived from a reduced shell; Kay (1968) in fact noted the close "approximation" of a single juliid valve to the reduced shell of *Lobiger*. It is interesting to note that the node at which shell reduction occurred can be easily shifted in each tree to accommodate the Juliidae (Figs. 1-8, arrows); if this is done, shell reduction becomes prerequisite to shell loss in two more topologies (Figs. 3-4), with reflective shifts from branched to linear character state trees.

NOTASPIDEAN OPISTHOBRANCHS

Willan (1987) published one of the first phylogenetic analyses involving opisthobranchs, in his investigation of the Notaspidea (= Pleurobranchomorpha, "side-gilled slugs"). Here again, condition of the shell has played a traditional role in classification (Boss, 1982; Marcus, 1984, 1985), including forms with external cap-shaped shells (Umbraculacea), and those with reduced or absent


11
Outgroup
3
Tylodina
Anidolyta
o
Umbraculum
PLEUROBRANCHACEA
12
Outgroup
3
Tylodina
Anidolyta
o
Umbraculum
PLEUROBRANCHACEA
13
Outgroup
Umbraculum
Tylodina
3
Anidolyta
PLEUROBRANCHACEA
14
Outgroup
Umbraculum
Tylodina
3
Anidolyta
PLEUROBRANCHACEA
15
Outgroup
UMBRACULACEA
PLEUROBRANCHAEINAE
Bathyberthella
2
Berthella
Pleurobranchus
Berthellina
Pleurehdera
16
Outgroup
UMBRACULACEA
PLEUROBRANCHAEINAE
Pleurobranchus
2
Berthella
Bathyberthella
Berthellina
Pleurehdera
17
Outgroup
UMBRACULACEA
PLEUROBRANCHAEINAE
Bathyberthella
2
Pleurobranchus
Berthella
Berthellina
Pleurehdera

Figs. 11-17. Notaspidean opisthobranch test data results. Figs. 11-14. Most-parsimonious tree topologies, basal region. Figs. 15-17. Same, top region. (2, shell internalization; 3, shell decalcification; •, shell reduction; ••, shell loss; o, reversal to shell calcified (character 3); arrow, position of shell internalization (character 2) if Pleurobranchaeinae are included; double slash, demarcation between basal and top tree regions).

shells (Pleurobranchacea: Pleurobranchidae: Pleurobranchinae [reduced in some] and Pleurobranchaeinae [lost in all]).

Willan's (1987: tables 3-5) original dataset included characters of the shell, mantle cavity, head-foot, digestive system, and reproductive system. The 57 characters in 11 genus-level taxa (Willan, 1987: table 5) included four binary shell characters: (1) 0 = present, 1 = absent; (2) 0 = external, 1 = internal; (3) 0 = calcified, 1 = uncalcified; and (11) 0 = mantle and shell subequal in size, 1 = mantle larger than shell. While characters 2 and 3 are attributes commonly noted in reduced shells (see above), characters 1 and 11 are expressions of shell loss and reduction, respectively, and were the only ones manipulated here. Following minor modifications to facilitate the parsimony-based Hennig86 analysis (see Notes in Table 2), the resulting list of 41 characters (Table 2) was subjected to the exhaustive Hennig86 algorithm, *ie** (implicit enumeration).

Each analysis produced the same number of trees (12 MPTs) of comparable length and tree statistics, with repetitive topology in two regions. The basal part of the tree, including the taxa of traditional Umbraculacea (*Tylodina*, *Anidolyta*, and *Umbraculum*), appeared in four topologies of three MPTs each (Figs. 11-14). Traditional Tylodinidae (*Tylodina* + *Anidolyta*) and Umbraculacea formed monophyletic clades in only one topology or three MPTs (25%) each (Figs. 13 and 11, respectively).

The "top" of the tree, comprising the eight taxa of Pleurobranchacea (and Pleurobranchidae) consistently placed the two traditional subfamilies, Pleurobranchaeinae and Pleurobranchinae, as monophyletic sister-groups. The three taxa of Pleurobranchaeinae (*Pleurobranchella* + *Pleurobranchaea* + *Euselenops*) formed a consistent monophyletic clade, hereafter combined as Pleurobranchaeinae. The five taxa of Pleurobranchinae (*Bathyberthella* + *Berthella* + *Pleurobranchus* + *Berthellina* + *Pleurehdera*) appeared in three topologies of four MPTs each (Figs. 15-17). Within this, *Berthellina* + *Pleurehdera* formed another consistent monophyletic clade (traditionally unnamed).

Shell absent (character 1 = 1) was consistently

Table 2. Notaspidean taxon list (with shell condition), character list, and data matrix (based on Willan, 1987).

TAXON LIST
Gastropoda
Heterobranchia
Opisthobranchia
Notaspidea
Umbraculacea
Fam. Tylodinidae
Tylodina - present, external, uncalcified
Anidolyta - present, external, uncalcified
Fam. Umbraculidae
Umbraculum - present, external, calcified
Pleurobranchacea
Fam. Pleurobranchidae
Subfam. Pleurobranchinae
Pleurobranchus - present, internal, uncalcified
Berthella - present, internal, uncalcified
Bathyberthella - present, internal, uncalcified
Pleurehdera - reduced, internal, uncalcified
Berthellina - reduced, internal, uncalcified
Subfam. Pleurobranchaeinae
Pleurobranchella - absent
Pleurobranchaea - absent
Euselenops - absent

CHARACTER LIST
0. Dummy all-zero.
1. Shell: 0, present; 1, absent (coding reversed).
2. Shell: 0, external; 1, internal.
3. Shell: 0, calcified; 1, uncalcified.
4. Periostracum: 0, smooth; 1, rough or lamellate.
5. Muscle scar: 0, incomplete; 1, intermediate suspensor present; 2, complete.
6. Shell shape: 0, circular; 1, rectangular.
7. Shell location: 0, central; 1, anterior; 2, posterior (Willan character 9, coding adjusted).
8. Shell size relative to body size: 0, large; 1, medium; 2, small (Willan character 10).
9. Shell size relative to mantle size: 0, subequal; 1, mantle larger than shell (Willan character 11).
10. Mantle: 0, smooth; 1, pustulose; 2, puckered (Willan character 12).
11. Mantle spicules: 0, absent; 1, present (Willan character 13).
12. Mantle border, anteriorly: 0, entire; 1, weakly emarginate; 2, deeply cleft (Willan character 14).
13. Mantle margin: 0, entire; 1, slightly crenulate; 2, deeply serrate (Willan character 16).
14. Mantle and oral veil: 0, separate; 1, fused (Willan character 18).
15. Oral tentacles: 0, separate; 1, joined by oral veil (Willan character 21).
16. Oral veil width relative to body: 0, very narrow; 1, narrow; 2, moderately broad; 3, very broad (Willan character 22).
17. Oral veil papillae: 0, absent; 1, present along anterior edge (Willan character 23).
18. Rhinophores: 0, separated; 1, together, unfused; 2, together, fused (Willan character 24).
19. Pedal gland: 0, absent; 1, present (Willan character 27).
20. Gill location: 0, well back, posterior right; 1, posterior right; 2, from left corner to posterior midline (Willan character 31).
21. Gill attachment, extent: 0, half length; 1, less than half length; 2, almost entire length (Willan character 32).
22. Gill rachis: 0, smooth; 1, pustulose (Willan character 33).
23. Anus relative to gill basement membrane: 0, at middle; 1, in front of hind end; 2, above hind end; 3, well behind gill (Willan character 34).
24. Median buccal gland: 0, absent; 1, present (Willan character 38).
25. Radular rachidian teeth: 0, present; 1, absent (Willan character 39; coding reversed).
26. Radular lateral teeth, denticle at base: 0, absent; 1, present (Willan character 40).
27. Radular lateral teeth: 0, not lamellate; 1, lamellate (Willan character 42).
28. Labial cuticle: 0, two separate thickenings (jaws); 1, continuous thickened ring (Willan character 43; coding reversed).
29. Mandibular elements: 0, oval or polygonal; 1, cruciform (Willan character 44; coding reversed).
30. Mandibular elements, blades: 0, denticulate; 1, smooth (Willan character 45; coding reversed).
31. Reproductive condition: 0, monaulic; 1, diaulic; 2, triaulic (Willan character 46).
32. Penial autospermal groove: 0, present; 1, absent (Willan character 48; coding reversed).
33. Penis location: 0, at base of right oral tentacle; 1, anterior midline; 2, on right side in front of gill (Willan character 49; coding adjusted).
34. Penis: 0, non-protrusible; 1, protrusible (Willan character 50).

(Continued)

Table 2. (continued)

35. Allosperm receptacles: 0, two; 1, one (Willan character 52; coding reversed).
36. Receptaculum seminis, origin: 0, low; 1, high (Willan character 53; coding reversed).
37. Prostate gland: 0, surrounding autosperm canal; 1, absent; 2, present as distinct organ (Willan character 54; coding adjusted).
38. Penial gland: 0, absent; 1, present (Willan character 55).
39. Penial sack: 0, absent; 1, present (Willan character 56).
40. Vas deferens, coiling within penial sack: 0, absent; 1, present (Willan character 57).

Notes: Changes to Willan's original coding were:
1. Autapomorphies were removed (characters 7, 15, 19, 20, 25, 26, 28, 30, 35, 36, 41, 47, and 51).
2. Inexplicably, some character states were originally numbered 0-1-2-etc., while others were numbered 1-2-3-etc. The latter characters (3, 5, 8-12, 14, 16, 17, 22, 24, 31-34, 39, 40, 45, 46, 49, 52, 54, and 55) were renumbered here so that the most plesiomorphic state was 0. With this accomplished, character 17 became all 0's, so was eliminated here.
3. In comparing the original coding scheme (Willan, 1987: table 4) with the character list (including plesiomorphic and apomorphic states), some of Willan's apomorphic character states were found inappropriately coded as 0. Coding was here adjusted (usually simply reversed) for characters 1, 9, 37, 39, 43, 44, 45, 48, 49, 52, 53, and 54. Following this recoding, character 37 became an autapomorphy and was eliminated.
4. Some taxa were originally listed as having multiple character states. These were coded here with the most plesiomorphic state. When this was done, characters 8 and 29 became all 1's and dashes (inapplicable), so were eliminated.
5. A probable error was noticed in one of the original shell characters (character 3, shell calcified/uncalcified), where the text claimed "only *Umbraculum* calcifies its shell to any degree" (Willan, 1987: 220) while *Umbraculum* and all other taxa except *Pleurobranchus* and *Bathyberthella* were originally coded as uncalcified. This character was recoded here according to Willan's statement, with the outgroup and *Umbraculum* as calcified (0) and all remaining taxa as uncalcified (1; although Marcus, 1985, noted that the shell of *Tylodina* includes a calcified part); the last three taxa in the matrix were coded here as "inapplicable" (-).
6. An all-zero outgroup was used, and an all-zero character 0 was added.

DATA MATRIX

Outgroup	00000000000000000000000000000000000000000
Tylodina	0001010000000000100000000200101—00201-0000
Anidolyta	0001100000000010100000000201011—10201-0000
Umbraculum	0000120010000200—10220301001—-01—-2000
Berthella	00110010000110011021110011000112101111100
Pleurobranchus	00110010002120011021111211000101101111000
Berthellina	00110011210100011020110211010112101000100
Pleurehdera	00110011110-00011021110211110102101010100
Bathyberthella	00110010000000011021110211000002101010000
Pleurobranchella	01--------10- 01121001101110000011011- 2011
Pleurobranchaea	01--------10- 01121011101100000011011- 2011
Euselenops	01--------00- 0113101111111000011101101000

synapomorphic for Pleurobranchaeinae (Figs. 15-17). Shell reduced (character 9 = Willan's character 11 = 1) was a consistent synapomorphy for the *Berthellina* + *Pleurehdera* clade, and was never prerequisite to shell loss (consistently a branched character state tree as in that given for Figs. 3-8). Shell internalization (character 2 = 1) was synapomorphic for Pleurobranchinae, but the sister clade, Pleurobranchaeinae, was coded "?" for this character, so the character state change could be a synapomorphy for Pleurobranchacea (Figs. 15-17, arrows). Shell decalcification (character 3 = 1) was less definitive, occurring below *Tylodina* in all top-region topologies (and synapomorphic for a large clade above *Umbraculum* in two topologies, Figs. 13-14), but requiring reversals in *Umbraculum* in two topologies (Figs. 11-12).

Binary Coding and Multistate Unordered Coding. These two analyses produced 12 MPTs of 76 steps (CI 0.72, RI 0.73). The Multistate Unordered Analysis combined characters 1 and 9 into one character: 0 = present (and subequal in size relative to mantle), 1 = reduced (in size relative to mantle), and 2 = absent. This replaced character 1, and character 9 was eliminated from the data matrix.

Multistate Ordered Coding. This analysis used the same data matrix as Multistate Unordered Coding, but the analysis was run with character 1 (only) ordered. The resulting 12 MPTs were of 77 steps (CI 0.71, RI 0.73).

Uncoded/Mapped. This analysis used the binary dataset in Table 2 but with characters 1 and 9 deactivated within Hennig86. The resulting 12 MPTs were of 74 steps (CI 0.71, RI 0.72).

Summary. Regardless of coding alternative, there was no change in resultant topologies using the notaspidean dataset (although the tree length and statistics did vary slightly). Interpretation of monophyletic clades and evolution of shell reduction/loss therefore did not change either.

SIGMURETHRAN PULMONATES

Like opisthobranchs, pulmonates include representatives with full, reduced, or absent shells; those with reduced shells are called either semislugs or slugs according to degree of reduction of the visceral mass (slugs having completely internalized shells, and semislugs having external shells that are too small to retract into; T. Pearce, pers. comm.; those lacking shells are also called slugs; Solem, 1974). Unlike in opisthobranchs, however, the degree of shell reduction does not play a traditional role in classification. The shells of land slugs have been judged of limited taxonomic value because of their variability (Reuse, 1983), and because of the convergence "inherent in" reduced shells (Solem, 1978). Terrestrial gastropods therefore present a similar-but-different case for this analysis.

Unfortunately, no robust phylogenetic analysis including a dataset involving all shell morphotypes (present/reduced/absent) suitable for this manipulation has been published. The best available data come from Tillier's (1989) massive anatomical monograph of Stylommatophora, which presented character coding for taxa that were translatable into a data matrix for this demonstration. Tillier's work included nearly 200 taxa (Tillier, 1989: appendix A), but only 17 characters (Tillier, 1989: appendix E) from relatively few organ systems (digestive, excretory, and nervous). To reduce the data matrix to more-normal proportions of characters and taxa, a subset of taxa was chosen for this analysis. Solem (1974) noted that only the Sigmurethra includes fully shelled forms, semislugs, and slugs, so for this analysis, representatives of the sigmurethran subgroup Aulacopoda were selected from Tillier's data. The final dataset included two suprafamilial groups, six families, and 22 genus-level ingroup taxa plus an all-zero outgroup (Table 3). Of the six families, one contains snails only, three contain snails plus either slugs or semislugs (or both), and two contain slugs or semislugs only. Tillier's 17 original characters (Tillier, 1989: appendix E; as clarified by Emberton and Tillier, 1995) were transformed into cladistic characters as noted in Table 3 (see Notes).

No shell reduction character was originally coded by Tillier, although taxa were indicated as "semislug" or "slug" where applicable (Tillier, 1989: appendix B). For this analysis, four character states were used to code the shell as: fully present, reduced (semislug), reduced (slug), and absent (determined in part from familial descriptions in Boss, 1982). Two reduced categories were used to preserve the distinction between the shells of semislugs and slugs, assuming (perhaps in oversimplification) that the shell's condition in slugs is further reduced from that in semislugs. The lists of taxa (22) and characters (20, with shell reduction in binary form) and the data matrix appear in Table 3.

Hennig86's algorithm, *mhennig** (multiple passes without branch-swapping), was used for all analyses (more robust algorithms completed, but produced extraordinarily large numbers of trees). Because of the extreme variability in the results of this analysis, repeating topologies could not be identified. In general, suprafamilial and familial groups were not supported, and because the focus of this demonstration lies in the pattern(s) of character evolution implied by the various results, only examples and character state trees will be presented.

Binary Coding. Analysis of the dataset produced a single MPT (Fig. 18) of 76 steps (CI 0.46, RI 0.57). Shell reduction (character 2 = 1 or 2) occurred in parallel three times. Two of these paths were direct unmodified changes to slug- (character 2 = 2) or semislug-type (character 2 = 1) reduction. The third was a path through semi-slug type reduction to two changes to slug-type reduction and one to shell loss (character 1 = 1).

Multistate Unordered Coding. This analysis combined binary characters 1 and 2 to form a single multistate shell character: 0 = present, 1 = reduced (semislug), 2 = reduced (slug), 3 = absent. The combined character replaced character 1; character 2 was eliminated. The algorithm produced a single MPT of exactly the same topology, length, and statistics as that produced in Binary Coding (Fig. 18).

Multistate Ordered Coding. This analysis used the same modified dataset as Multistate Unordered Coding, but the analysis was run with character 1 (only) ordered. This scenario thus presupposed that shell reduction occurs in this lineage in the following linear order: semislug (reduced) to slug (reduced) to shell absent. The analysis produced seven MPTs of 77 steps (CI 0.45, RI 0.57). Six of the seven trees produced a character state tree and topology similar to that in Fig. 19. The character state tree reflected the linear ordering, but included one reversal from semislug-type reduction back to unreduced. The seventh MPT was similar except lacked the reversal, through relocating the shelled snails to the basal region of the tree.

Uncoded/Mapped. With characters 1 and 2 deactivated from the data matrix in Table 3, a single MPT was produced of 70 steps (CI 0.45, RI 0.55). When mapped on the tree, shell reduction characters produced a generally more complex pattern than in the previous methods (Fig. 20). Reduction occurred three times in parallel, two of these being direct unmodified changes to slug-type reduction. The third pathway was semislug-type reduction leading to slug-type reduction once, shell loss once, and reversals to unreduced shells twice.

Summary. (1) Although Binary and Multistate Unordered Coding produced the same result, Multistate Ordered Coding produced more trees (all different from the previous); Uncoded/Mapped again produced one tree, but again of a unique topology. (2) Suprageneric groups (*i. e.*

traditional superfamilies and families) were inconsistent, therefore monophyletic clades differed drastically depending on coding alternative used. (3) Shell reduction characters formed synapomorphies for at least one multi-taxon clade in all trees, but the supported clades were again dependent upon coding alternative. (4) The hypothesis of evolution of shell reduction and loss (expressed in character state trees) was highly dependent upon coding alternative. Ordering of the character states (unreduced to semislug-type reduction to slug-type reduction to loss) never occurred except when forced by Multistate Ordered Coding.

DISCUSSION

WHAT IS THE "CORRECT" METHOD?

Each of the above trials illustrated four ways of handling one traditional character in a cladistic dataset. The results ranged from differing greatly among methods (pulmonates), to differing slightly (sacoglossans), to not differing at all (notaspideans). The degree of difference corresponded here to what qualitatively appears to be relative strength of the total dataset (especially of non-shell characters therein), expressed by (in pulmonate, sacoglossan, and notaspidean datasets, respectively) (1) the Hennig86 algorithm that would readily resolve (*m**, *m*bb**, and *ie**), (2) range of CI (0.36-0.37, 0.40-0.41, and 0.71-0.72), (3) the amount of overlap among the trial results (very little, some, and total), and (4) the amount of change caused by deactivation of the shell reduction/loss characters (much shorter, but more MPTs; slightly shorter, slightly more MPTs; and no change). Interestingly, one other dataset manipulated in this same manner reacted like the notaspidean dataset - *ie** algorithm, total overlap of results, no change caused by deactivating the character in question. This was the truncatellid gastropod dataset published by Rosenberg (1996), manipulating a gill character originally coded as 0 = present, 1 = reduced, and 2 = absent; this result can also be attributed to the strength of the original dataset.

Binary versus Multistate Coding. Hauser and Presch (1991) theorized that an unordered analysis containing multistate characters should produce exactly the same results as a binary analysis. This was universally true here for all three datasets.

Binary coding of shell reduction/loss characters requires the use of missing character states (= unknown or inapplicable question marks) in the dataset. According to the literature, these can increase the number of MPTs (Wilkinson, 1995) or can cause other missing-data problems attributed to long-distance influence (Maddison, 1993). However in these test datasets, the presence of "?"s did not appear to induce problems, perhaps because these were "safe circumstances" as described by Maddison (1993: 579) where the "inapplicable" regions of the trees were confined to a single taxon (pulmonates) or clade (sacoglossans and notaspideans).

Other arguments against Binary Coding include: (1) binary characters could be redundant or not completely independent (Pimentel and Riggens, 1987; Maddison, 1993), and (2) if there is in fact evidence for an ordered transformational character, this evidence will be forfeited through binary coding and can be lost to the result (Pogue and Mickevich, 1990; Lipscomb, 1994).

Ordered versus Unordered. Hauser and Presch (1991) reanalyzed 27 published datasets to test the results of ordered versus unordered characters; their ordered analyses affected all multistate characters in each dataset, rather than only one as in the test cases here. In agreement with their results, ordering did not demonstrably improve clade resolution, and the number of trees was not necessarily reduced by ordering. Slowinski (1993) reanalyzed 21 published matrices and found similar results: unordered analyses resulted in overall greater resolution and greater congruence. Hauser and Presch (1991: 253) noted that "the effect of ordered characters ... is, in part, based on their interaction with other characters in the datamatrix" so that each individual change to a dataset affects multiple levels. Nevertheless, achieving the best resolved and smallest number of trees out of a dataset is not acceptable rationale for ordering characters. Ordering restricts possible character state transformations and requires independent, corroborating evidence that such a pattern occurred (or that others did not) (*i. e.* ontogeny; Hauser and Presch, 1991).

Slowinski (1993) noted that linear ordering (the most common method) does not always convey the best possible assumption of transformation for multistate characters with four or more states. Here, linear ordering of the shell reduction/loss multistate character in the pulmonate dataset (Fig. 19) presupposed that semislug-type shell reduction preceded slug-type reduction, which in turn preceded shell loss. In all other pulmonate results (Figs. 18, 20), this was not the case as evidenced by branched character state trees; shell loss was most often preceded only by semislug-type reduction.

Choosing an unordered analysis, or "letting the algorithm decide," clearly makes the fewest *a priori* assumptions, and will in fact reveal an ordered transformation series if it is part of the MPT(s). Mickevich and Weller (1990) agreed in part (advocating ordering in general), noting that the pattern of character state change revealed by an unordered analysis can test the validity of a hypothetical ordered transformation series. In the case of the sacoglossan analysis here, the 26 MPTs produced by ordering were a subset of those (56) produced by unordering; the ordered transformation was present in the two most frequently-

Table 3. Sigmurethran pulmonate taxon list (with shell condition), character list, and data matrix. Generic abbreviations (also used in Figs. 18-20) derived from superfamilial group, family, and genus names. Families and generic contents of families are as according to Vaught (1989); suprafamilial groupings are as according to Boss (1982) and South (1992)

TAXON LIST
Gastropoda
Pulmonata
Sigmurethra
Aulacopoda
 Arionoidea
  Fam. Arionidae
   Hemphillia (ArArHm) - reduced, semislug
   Aphallarion (ArArAp) - reduced, slug
   Oopelta (ArArOo) - reduced, slug
  Fam. Philomycidae
   Philomycus (ArPhPh) - absent, slug
  Fam. Endodontidae
   Thaumatodon (ArEnTh) - present, snail
   Phrixgnathus (ArEnPh) - present, snail
 Limacoidea
  Fam. Helicarionidae
   Hemiplecta (LiHeHe) - present, snail
   Microparmarion (LiHeMi) - reduced, semislug
   Cystopelta (LiHeCy) - reduced, semislug
   Mariaella (LiHeMa) - reduced, slug
  Fam. Urocyclidae
   Trochozonites (LiUrTr) - present, snail
   Trochonanina (LiUrTc) - present, snail
   Acantharion (LiUrAc) - reduced, semislug
   Mesafricarion (LiUrMe) - reduced, semislug
   Atoxon (LiUrAt) - reduced, slug
   Elisolimax (LiUrEl) - reduced, slug
  Fam. Zonitidae
   Trochomorpha (LiZoTr) - present, snail
   Zonites (LiZoZo) - present, snail
   Phenacolimax (LiZoPh) - reduced, semislug
   Vitrinopsis (LiZoVi) - reduced, semislug
   Daudebardia (LiZoDa) - reduced, slug
   Plutonia (LiZoPl) - reduced, slug

CHARACTER LIST
0. Dummy all-zero.
1. Shell: 0, present; 1, absent.
2. Shell: 0, fully present; 1, reduced (semislug); 2, reduced (slug).
3. Buccal mass (BM): 0, spheroidal to ovoidal tending toward cylindrical (BM1); 1, clearly cylindrical (BM2).
4. Esophageal crop (OC): 0, absent (OC1); 1, separated from gastric crop by distinct portion of esophagus (OC2); 2, separated from gastric crop by simple constriction (OC3); 3, as in OC3 but extending forward to nerve ring (OC4).
5. Gastric crop (SC): 0, cylindrical (SC1); 1, median portion inflated (SC2); 2, funnelform, widening from esophagus to stomach (SC2'); 3, anterior region inflated (SC3).
6. Gastric pouch (PS): 0, joining gastric crop without any constriction, distinctly wider than crop (PS1); 1, joining gastric crop without any constriction, slightly wider or no wider than crop (PS2); 2, separated from gastric crop by constriction, distinctly wider than crop (PS2').
7. Intestine length (IL): 0, intestinal loops long, reaching level between distal limit of gastric pouch and middle of gastric crop (IL1); 1, intestine shorter, but loops distinct (IL2); 2, intestinal loops long, reaching proximally at least to level of distal limit of gastric pouch (IL2'); 3, intestinal loops reduced to almost flat sigmoid (IL3).
8. Ratio of kidney length:lung length (LR): 0, very short kidney, 0.45-0.7 (LR1); 1, 0.36-0.45 (LR2); 2, 0.7-1.0 (LR2'); 3, 0.25-0.36 (LR3); 4, very long kidney, 0.0-0.25 (LR4) [slugs and semislugs not scored, fide Emberton & Tillier, 1995: 203].
9. Degree of closure of ureter (UR): 0, no closed retrograde ureter = complete mesurethry (UR1); 1, closed ureter reaching at most lung top (UR2); 2, ureteric tube reaching point between lung top and pneumostome (UR3); 3, ureteric tube reaching pneumostome = fully closed = complete sigmurethry (UR4).
10. Internal morphology of kidney (RR): 0, either two distinct regions (distal one usually lacking lamellae) or three distinct regions (median one either lacking lamellae or with lamellae different in appearance from those in proximal) (RR1); 1, kidney homogenous in internal morphology, with lamellae reaching distal region and level of kidney pore (RR2).
11. Cerebral commissure length (CC): 0, greater than 1.1 times right cerebral ganglion width (CC1); 1, 0.9-1.1 times right cerebral ganglion width (CC2); 2, less than 0.9 times right cerebral ganglion width (CC3).

(Continued)

Table 3. (continued)

12. Right cerebropedal connective length (CPD): 0, longer than twice right cerebral ganglion width (CPD1); 1, 1-2 times right cerebral ganglion width (CPD2); 2, shorter than right cerebral ganglion width (CPD3).
13. Ratio of lengths of left:right cerebropedal connectives (CPR): 0, < 0.9 (CPR1); 1, 0.9-1.1 (CPR2); 2, 1.1-1.5 (CPR3); 3, 1.5-2.5 (CPR4).
14. Right pleural ganglion position (PLD): 0, closer to pedal than cerebral ganglion = hypoathroid (PLD1); 1, closer to cerebral than pedal ganglion = epiathroid (PLD2).
15. Left pleural ganglion position (PLG): 0, closer to pedal than cerebral ganglion = hypoathroid (PLG1); 1, closer to cerebral than pedal ganglion = epiathroid (PLG2).
16. Visceral ganglion position relative to median plane of pedal ganglia (VG): 0, on right side (VG1); 1, in middle (VG2); 2, on left side (VG3).
17. Right parietal and pleural ganglia (PAD): 0, separate (PAD1); 1, in contact or fused (PAD2).
18. Left parietal ganglion position (PAG): 0, in contact with left pleural, or closer to it than to visceral ganglion, and separated from both by distinct connective (PAG1); 1, closer to visceral than to left pleural ganglion, and separated from both by distinct connective (PAG2); 2, in contact with visceral ganglion only, and separated from left pleural by distinct connective (PAG3); 3, in contact or fused with both left pleural and visceral ganglia (PAG4).
19. Fusion of visceral ganglion (FG): 0, none (FG1); 1, with right parietal ganglion (FG2); 2, with left parietal ganglion (FG2'); 3, with both parietal ganglia (FG3).

Notes: Tillier's characters (Tillier, 1989: appendix E; as clarified by Emberton and Tillier, 1995) were transformed:
1. by changing original 1-2-2'-3 character codes (reflecting ordered character state changes, with primed states indicating branched character state trees) into linear 0-1-2-3 cladistic character states.
2. by coding any 0-states in the original matrix (unexplained by Tillier and called "eliminated" by Emberton and Tillier, 1995) as "-."
3. where more than one representative per genus was coded by Tillier, by combining them and choosing the most plesiomorphic state (but never "-") for the cladistic data matrix.

DATA MATRIX

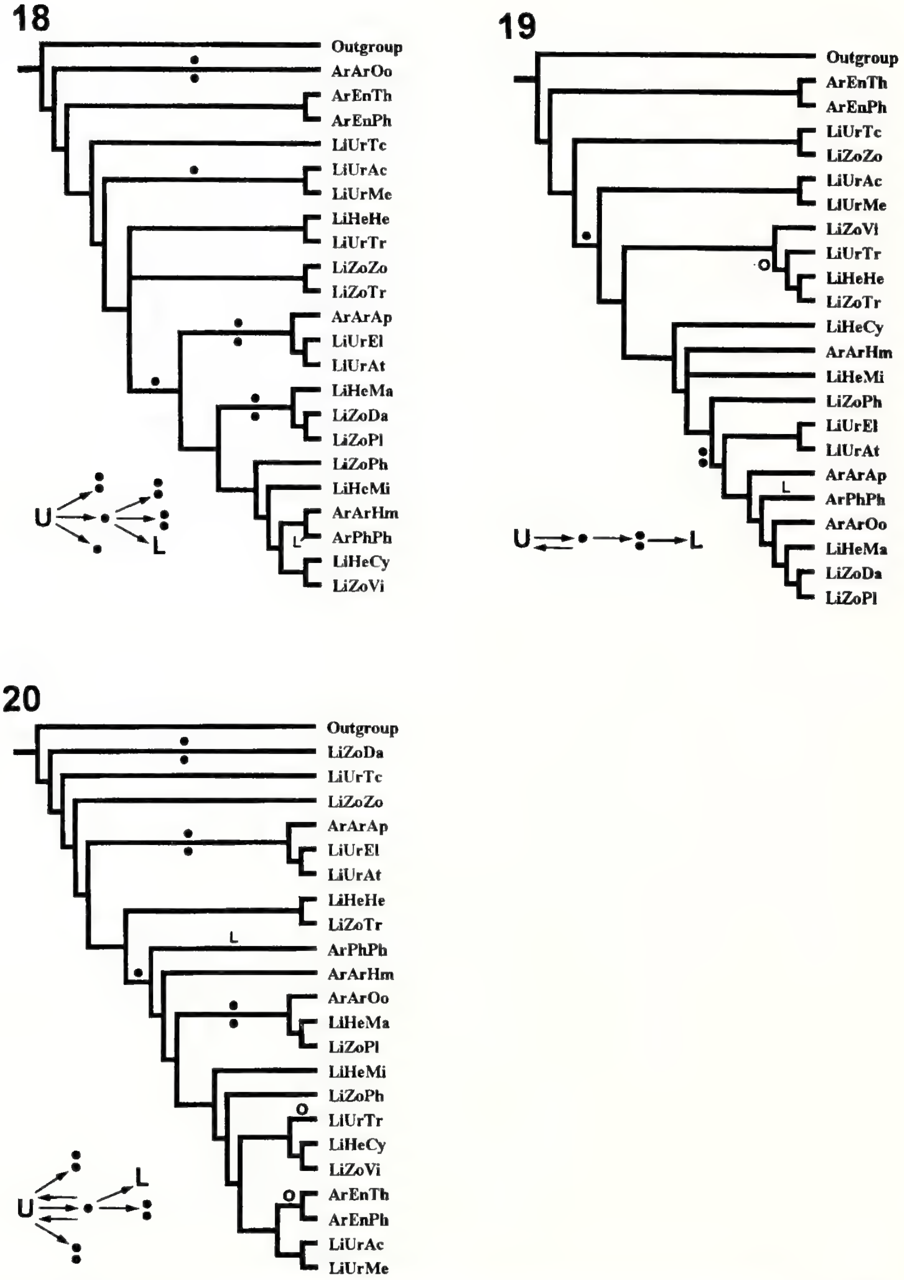
Outgrp	0000000000000000000
ArArHm	00100311-31121000123
ArArAp	00200002-31- - - - - - - -
ArArOo	00200000-31010000122
ArPhPh	01-00000-31221- - -1123
ArEnTh	00000311-10110110001
ArEnPh	0000030133021210010 1
LiHeHe	0000000123022100112 0
LiHeMi	00102-01231222 - 00133
LiHeCy	00100001-30220 - - 0133
LiHeMa	00202111231- - - - - - - -
LiUrTr	0000000113022200012 0
LiUrTc	0000000103011000113 0
LiUrAc	0010230023021100013 1
LiUrMe	00102300 - - -211001130
LiUrAt	00203 -12 - 31221001130
LiUrEl	00203 - 12231- - - - - - - -
LiZoTr	0000020123022111111 0
LiZoZo	0000020103120100113 0
LiZoPh	00100001- 31222001131
LiZoVi	00100001230 - - - - - - - -
LiZoDa	00210111- - -000001011
LiZoPl	00210111 - 31212002122

occurring topologies and 46% of the MPTs. Ordering only eliminated possible topologies from consideration.

Uncoded/Mapped Characters. *A posteriori* mapping of characters that one deems unusable is also *a priori* reasoning - these characters have been judged beforehand to play no role, or a conflicting role, in evolution of the group. If total evidence (usually combining molecular plus morphological characters in a single dataset) is the best accepted method of determining phylogenetic relationships (Kluge, 1989; de Queiroz *et al.*, 1995), then the same should also be preferred at the morphological level, *i. e.* incorporating as many morphological characters (including negative gain characters) as possible.

TRANSFORMING PHYLOGENETIC ANALYSES INTO CLASSIFICATIONS

Revising taxonomic classification is usually not the sole (nor primary) question being approached using cladistics. Nevertheless, it is always tempting to translate resultant tree(s) following an analysis. Much of the discussion


18
Outgroup
ArArOo
ArEnTh
ArEnPh
LiUrTc
LiUrAc
LiUrMe
LiHeHe
LiUrTr
LiZoZo
LiZoTr
ArArAp
LiUrEl
LiUrAt
LiHeMa
LiZoDa
LiZoPl
LiZoPh
LiHeMi
ArArHm
ArPhPh
LiHeCy
LiZoVi
L
U
L
19
Outgroup
ArEnTh
ArEnPh
LiUrTc
LiZoZo
LiUrAc
LiUrMe
LiZoVi
LiUrTr
LiHeHe
LiZoTr
LiHeCy
ArArHm
LiHeMi
LiZoPh
LiUrEl
LiUrAt
ArArAp
ArPhPh
ArArOo
LiHeMa
LiZoDa
LiZoPl
o
L
U
L
20
Outgroup
LiZoDa
LiUrTc
LiZoZo
ArArAp
LiUrEl
LiUrAt
LiHeHe
LiZoTr
ArPhPh
ArArHm
ArArOo
LiHeMa
LiZoPl
LiHeMi
LiZoPh
LiUrTr
LiHeCy
LiZoVi
ArEnTh
ArEnPh
LiUrAc
LiUrMe
L
o
o
U
L

Figs. 18-20. Pulmonate test dataset results, sample most-parsimonious cladograms and character state trees. Fig. 18. Binary and Multistate Unordered Coding. Fig. 19. Multistate Ordered Coding. Fig. 20. Uncoded/Mapped. See Table 3 for taxon abbreviations. (•, semislug-type reduction; ••, slug-type reduction; o, reversal to unreduced shell; L, shell loss; U, unreduced shell).

here might have centered on differences in, for example, the number of superfamilies required by one cladogram over another. But, abandoning taxonomic rank (as suggested by de Queiroz and Gauthier, 1992; Ponder and Lindberg, 1997; Roth, 1997), at least above ICZN-regulated family-level (ICZN, 1985), renders this discussion irrelevant. Above family-level, it is possible to refer to a clade without worrying about whether it is, for example, a subclass or an order. Abandonment of rank furthermore eliminates the need to erect meaningless "redundant categories" (reviewed by de Queiroz and Gauthier, 1992), *e. g.* an order Cylindrobullacea and family Cylindrobullidae for the monotypic clade defined only by the genus-level synapomorphies of *Cylindrobulla* (Jensen, 1996b).

If one chooses to do so, the number of recognized taxa derived from a cladogram depends on monophyletic clades (not grades or paraphyletic taxa; Bieler, 1990). As shown by these experimental manipulations, the choice of coding alternative can affect this result. Deciding which monophyletic clades to recognize is still a subjective step, and depends on synapomorphies and other node-defining character state changes. For example, in the sacoglossan trials, Placobranchacea was consistently supported here. Some of the resultant topologies resulted in a large number of taxa at a level equivalent to Placobranchacea, *e. g.* from Fig. 2: *Ascobulla, Volvatella*, Juliidae, and Oxynoidae. But whether these are recognized as five monophyletic clades, or four (*Ascobulla, Volvatella*, Juliidae, Oxynoidae + Placobranchacea), or three (*Ascobulla, Volvatella*, Juliidae + Oxynoidae + Placobranchacea), or two (*Ascobulla, Volvatella* + Juliidae + Oxynoidae + Placobranchacea), or one (all five combined) depends on at which node(s) sufficient support is recognized.

If a cladogram such as that in Fig. 2, suggesting a drastically new classification, is chosen as the preferred tree, the degree of confidence placed in the dataset and its coded characters should determine how to proceed. Is this a robust analysis, with strong corroborating support (*e. g.* high bootstrap or other statistical values; large number of characters), and where the cladogram shows little change with each added character or taxon? Or is this preliminary, with a cladogram that is likely to change topology dramatically as new data are obtained? If the former, then the result must be trusted, regardless of how closely the cladogram agrees with traditional classification or an initial hypothesis of the "true tree." If the latter, then construction of a hierarchial classification should be postponed awaiting further data.

CONCLUSIONS

1. Choosing characters and how to code them is the fundamental step in cladistics - and one not without subjectivity. Precise definition of negative gain characters, such as shell reduction and loss, is critical, requiring more "diligen[ce] in the detail and consistency of terminology" (Proctor, 1996: 145) than in the case of positive gain characters. How to code such characters is equally critical, and as was shown, different coding alternatives can (although not always) produce different cladograms, which can be translated into different classifications - a point made earlier by Pogue and Mickevich (1990).

2. How a data matrix is analyzed is less critical, and it is not the goal of this paper to recommend one method over another. Although Multistate Unordered Coding might be interpreted as having the most support (Pimentel and Riggens, 1987), in the absence of theoretical or procedural preference, the best alternative is to use multiple methods (agreed by Hauser and Presch, 1991; Kim, 1993; but not by Wilkinson, 1992) at least during the iterative stage of character development. As noted by Kim (1993: 335), "agreement among trees estimated by different methods lends greater credibility to the estimates." Ponder and Lindberg (1997) used this approach, including recoding of multistate characters in their dataset as binary characters. This is comparable to the method commonly used as an alternative to total evidence - running separate analyses of molecular versus morphological (*e. g.* Hillis *et al.*, 1996; Shaffer *et al.*, 1997) or behavioral versus morphological data (*e. g.* Prum, 1990), and comparing results generated by the different methods, perhaps also in combined format.

3. Homoplasy should be hypothesized from a phylogenetic analysis, not initially assumed. Negative gain characters, such as shell reduction and loss, have a high likelihood for homoplasy and as such might be preconceived as less informative than positive gain characters in a cladistic analysis. However, Sanderson and Donoghue (1996) showed that homoplasy in a cladogram (resulting in a low CI) can co-occur with a high level of confidence (expressed in that case by high bootstrap support values), which means that potentially homoplastic characters need not automatically be omitted.

Furthermore, homoplasy does not necessarily indicate error or noise. Numerous parsimony-based analyses present high levels of homoplasy (Sober, 1992; Foley, 1993), and many authors have considered homoplasy to "constitute the majority of evolutionary change during the course of evolution and diversification of lineages" (Armbruster, 1996: 227; see also Hennig, 1966, 1983; Gosliner and Ghiselin, 1984; Sluys, 1989b; Moore and Willmer, 1997). Parallelism is often invoked in interpretation of cladograms (*e. g.* Tassy, 1988; Sluys, 1989a; Erséus, 1990; Griswold, 1993; Jensen, 1996b; Salvini-Plawen and Steiner, 1996), absences are presented as synapomorphies (Ax, 1987; Müller and Wagner, 1991), and homoplasy occurs in nearly every cladistic study; in a reanalysis of 38 published data matrices, Maddison (1991: table 2) obtained consistency indices [CIs] ranging from 0.198-0.808 (mean = 0.515).

Wilkinson (1991) opined that parsimony methods fail not when homoplasy is rampant, but when homoplasy is based on misleading evidence - therefore, careful definition of characters and character states is the key. Cladistics is a tool - albeit a powerful one - in studying molluscan systematics; as such, it can help us study homoplasy. Including a limited number of negative gain characters (in conjunction with sufficient positive gain characters) can serve to highlight homoplasy against a background of homology on the final tree (Platnick, 1977), and is the strongest method of testing hypotheses of loss. A low CI-value does not (necessarily) mean that a final result is poor. In these cases, a homoplastic character is informative as long as its suggested transformation is not biologically impossible.

Armbruster (1996: 240) noted that "homoplasy is common in characters with ecological significance" and "characters of ecological importance are often of little systematic utility." These same statements can be said of shell reduction and loss in the Mollusca: "in virtually every major group of mollusks there are some species in which the shell has become reduced to a remnant" (Solem, 1974: 16). Inclusion of these characters wherever possible in analyses could lead to more rigorous documentation of the number and extent of "trends toward shell loss" throughout the phylum.

4. Revising taxonomic classification is only part of why we do cladistics, and might not be supportable from a given analysis. Cladistic analyses rarely generate fully resolved trees with all monophyletic taxa supported by strong synapomorphies. Several equally supported classifications can often be inferred from a single cladogram (Tassy, 1988), depending on the choice of monophyletic groups. Although new cladistic analyses (or reanalyses) are being regularly produced, a phylogenetic classification of all Mollusca still eludes us. In the interim, we must provisionally accept untested and even paraphyletic taxa in molluscan taxonomy, and avoid proposing unstable classifications that will change with the next analysis.

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Reproducibility of results in phylogenetic analysis of mollusks: a reanalysis of the Taylor, Kantor, and Sysoev (1993) data set for conoidean gastropods

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Abstract: Reanalysis of the Taylor, Kantor, and Sysoev (1993) data set on conoidean gastropods failed to reproduce their results. Taylor *et al.* found more than 900 trees of length 189; reanalysis yielded 32,700 trees of length 187. The number of trees they found was limited by the memory available on the computer used for the analysis. The Taylor *et al.* consensus tree omitted the stated outgroup *Benthobia* (Pseudolividae); reanalysis including the outgroup yielded 3,149 trees of length 193, in all of which *Benthobia* fell within the ingroup. Strict and majority-rule consensus trees differed considerably in topology from those with *Benthobia* excluded. Reanalysis excluding the hypothetical ancestor, whose character states Taylor *et al.* determined in part by ingroup analysis, yielded additional topologies of consensus trees. Only eight of 38 clades in the Taylor *et al.* tree appeared in all three strict consensus trees; 17 clades were not supported by any of the majority rule consensus trees. All three majority-rule consensus trees did support the transfer to Conidae by Taylor *et al.* of the turrid subfamilies Clathurellinae, Conorbinae, Oenopotinae, Mangeliinae, Daphnellinae, and Taraninae. This clade, however, did not appear in two of the strict consensus trees, so support for it is equivocal.

Additional problems with the analysis include incorrect character mappings, use of characters primarily from one organ system, conflicts between text and data matrix, choice of taxa, and inclusion of data from taxa not included in the cladistic analysis in formulating the classification. The Taylor *et al.* data set does not support strong inferences about conoidean phylogeny, and there is not yet convincing evidence for abandoning the traditional classification of the group. Nonetheless, their data are an immensely valuable contribution to be built on as information about conoidean taxa, characters, organ systems, and outgroups accumulates.

Key words: cladistics, critique, Conoidea, anatomy, classification

Taylor, Kantor, and Sysoev (1993) presented a enormous body of anatomical data on conoidean gastropods, along with a phylogenetic analysis of their data. The phylogenetic tree they presented is reproduced here (Fig. 1), and will be referred to hereafter as the TKS tree, from the authors' initials. Based on their analysis, Taylor *et al.* introduced a revised classification of the superfamily Conoidea, which differs in a number of respects from the traditional classification that recognizes three families, Conidae, Turridae, and Terebridae. In particular, the turrid subfamilies Clathurellinae, Conorbinae, Oenopotinae, Mangeliinae, Daphnellinae, and Taraninae were transferred to Conidae, and Drilliidae, Pseudomelatomidae, and Strictispiridae, formerly subfamilies of Turridae, were elevated to familial status. Kohn and McLean (1994) critiqued the classification, but did not try to replicate the analysis. I attempted to reproduce the results of Taylor *et al.*, by reanalyzing their data, but was unable to do so. I found many more, and shorter trees, than they did, but because they gave insufficient detail about methods, it was difficult to tell why we had gotten different results.

There is always a danger that such a reanalysis will be viewed as a personal attack, although the debate between Bieler (1990) and Haszprunar (1990) over Haszprunar's (1988) "clado-evolutionary" classification of the Gastropoda shows that salutary results are possible. It was therefore with some trepidation that I approached Dr. John Taylor and Dr. Yuri Kantor, at a much later date than I should have (July 1998), to ask for clarification of their methods. They asked me to keep in mind that the analysis published in 1993 was performed in 1991, and that computing power and the sophistication of cladistic methods have both advanced considerably in the intervening seven years. With their cooperation, I have been able to include their comments about specific methodological points.

The question of whether the results of a phylogenetic analysis can be reproduced can be addressed at several levels. Given the same data and the same assumptions, can the results be replicated? How much do the results change with different assumptions? These are essentially questions of the mechanics of the analysis and its internal consistency. Equally important, but much harder to answer: would a

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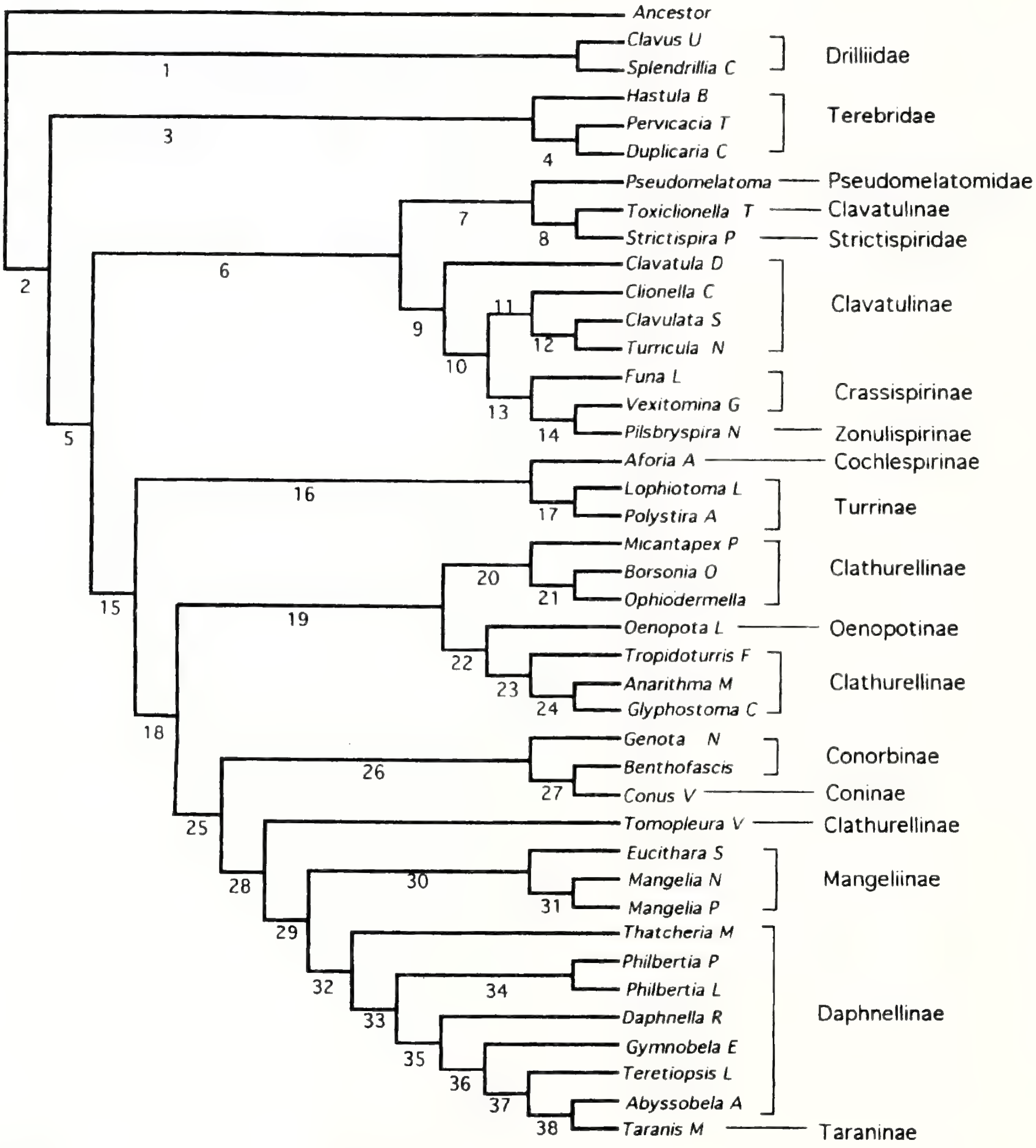
Ancestor
Clavus U
Splendrillia C
Drilliidae
1
Hastula B
Pervicacia T
Duplicaria C
Terebridae
3
4
2
Pseudomelatoma
Pseudomelatomidae
Toxiclionella T
Clavatulinae
Strictispira P
Strictispiridae
7
8
6
Clavatula D
Clionella C
Clavulata S
Turricula N
Clavatulinae
9
10
11
12
Funa L
Vexitomina G
Crassispirinae
Pilsbryspira N
Zonulispirinae
13
14
5
Aforia A
Cochlespirinae
Lophiotoma L
Polystira A
Turrinae
16
17
Micantapex P
Borsonia O
Ophiodermella
Clathurellinae
20
21
19
15
Oenopota L
Oenopotinae
Tropidoturris F
Anarithma M
Glyphostoma C
Clathurellinae
22
23
24
18
Genota N
Benthofascis
Conorbinae
Conus V
Coninae
26
27
25
Tomopleura V
Clathurellinae
Eucithara S
Mangelia N
Mangelia P
Mangeliinae
28
30
31
29
Thatcheria M
Philbertia P
Philbertia L
Daphnella R
Gymnobela E
Teretiopsis L
Abyssobela A
Daphnellinae
Taranis M
Taraninae
32
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37
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Fig. 1. TKS tree (50% majority-rule consensus tree from Taylor *et al.*, 1993: fig. 27). Names at the right show the new classification proposed by Taylor *et al.* Taxa are, in alphabetical order [with abbreviations in brackets]: *Abyssobela atoxica* Kantor & Sysoev, 1986 [*Abyssobela* A], *Aforia abyssalis* Sysoev & Kantor, 1987 [*Aforia* A], *Anarithma metula* (Hinds, 1843) [*Anarithma* M], Ancestor (hypothetical) outgroup, *Benthobia* outgroup, *Benthofascis biconica* (Hedley, 1903) [*Benthofascis*], *Borsonia ochraea* Thiele, 1925 [*Borsonia* O], *Clavatula caerulea* (Weinkauff, 1875) [*Clavatula* S; *Clavatula* C in Figs. 2-7], *C. diadema* Kiener, 1840 [*Clavatula* D], *Clavus unizonalis* (Lamarck, 1822) [*Clavus* U], *Clionella sinuata* (Born, 1778) [*Clionella* C], *Conus ventricosus* Gmelin, 1791 [*Conus* V], *Daphnella reeveeana* (Deshayes, 1863) [*Daphnella* R], *Duplicaria colorata* Bratcher, 1988 [*Duplicaria* C], *Eucithara stromboides* (Reeve, 1846) [*Eucithara* S], *Funa latisinuata* (E. A. Smith, 1877) [*Funa* L], *Genota nicklesi* Knudsen, 1952 [*Genota* N], *Glyphostoma candidum* (Hinds, 1843) [*Glyphostoma* C], *Gymnobela emertoni* (Verrill and Smith, 1884) [*Gymnobela* E], *Hastula bacillus* (Deshayes, 1859) [*Hastula* B], *Lophiotoma leucotropis* (Adams and Reeve, 1850) [*Lophiotoma* L], *Mangelia nebula* (Montagu, 1803) [*Mangelia* N], *M. powisiana* (Dautzenberg, 1887) [*Mangelia* P], *Micantapex parengonius* Dell, 1956 [*Micantapex* P], *Oenopota levidensis* (Carpenter, 1864) [*Oenopota* L], *Ophiodermella inermis* (Hinds, 1843) [*Ophiodermella*], *Pervicacia tristis* (Deshayes, 1859) [*Pervicacia* T], *Philbertia linearis* (Montagu, 1803) [*Philbertia* L], *P. purpurea* (Montagu, 1803) [*Philbertia* P], *Pilsbryspira nymphia* (Pilsbry and Lowe, 1932) [*Pilsbryspira* N], *Polystira albida* (Perry, 1811) [*Polystira* A], *Pseudomelatoma penicillata* (Carpenter, 1864) [*Pseudomelatoma*], *Splendrillia chathamensis* Sysoev and Kantor, 1989 [*Splendrillia* C], *Strictispira paxillus* (Reeve, 1845) [*Strictispira* P], *Taranis moerchii* (Malm, 1863) [*Taranis* M], *Teretiopsis levicarinata* Kantor and Sysoev, 1989 [*Teretiopsis* L], *Thatcheria mirabilis* Angas, 1877 [*Thatcheria* M], *Tomopleura reevii* (C. B. Adams, 1850) [*Tomopleura* V], *Toxiclionella tumida* (Sowerby, 1870) [*Toxiclionella* T], *Tropidoturris fossata notialis* Kilburn, 1986 [*Tropidoturris* F], *Turricula nelliae spurius* (Hedley, 1922) [*Turricula* N], and *Vexitomina garrardi* (Laseron, 1954) [*Vexitomina* G]. [Reproduced with permission from the *Bulletin of the Natural History Museum (London)*.]

different data set, with different characters or different representative taxa, lead to similar phylogenetic inferences? In principle, it should, because there is only one true tree. If a phylogenetic analysis fails in either internal or external consistency, then it is not a good basis for classification. Because I am not an expert on conoidean anatomy, I have considered mainly the internal consistency of the Taylor *et al.* data set, rather than attempting to formulate a revised data set.

METHODS

Parsimony analysis was performed using PAUP 3.1.1 (Swofford and Begle, 1993) on an Apple Macintosh Centris 660AV computer. Taylor *et al.* used a Macintosh SE (pers. comm.) running PAUP 3.0. Three versions of the data set were studied: Analysis I, the original data matrix, exactly as presented by Taylor *et al.*; Analysis II, the original matrix excluding the outgroup *Benthobia* (Pseudolividae); and Analysis III, the original matrix excluding *Benthobia* and the hypothetical ancestor.

The heuristic search option of PAUP was used, with all characters unordered and equally weighted and search settings as follows: Random addition sequence (10 replicates); tree-bisection-reconnection branch-swapping performed; MULPARS in effect; steepest descent in effect; branches having maximum length zero collapsed to yield polytomies; topological constraints not enforced; trees unrooted; no trees in memory at start of run. Taylor *et al.* (1993: 152) also performed a heuristic search but did not state any of the settings. They were unclear about ordering of characters and did not mention weighting.

Strict and majority rule consensus trees were computed for the minimal trees for each set of taxa analyzed, along with partition lists showing clades found in one or more trees and their frequency of occurrence. MacClade 3.0.1 (Maddison and Maddison, 1992) was used to determine the length of particular trees, based on the original matrix, again with all characters unordered and equally weighted.

The following tree statistics were calculated: consistency index (CI), homoplasy index (HI), rescaled consistency index (RC), and retention index (RI).

RESULTS

Within each run, only one to four of ten replicates were completed before PAUP's maximum trees limit of 32,700 was reached, terminating the run. Therefore each analysis was run four times, with different random starting seeds to give different input orders of taxa. In some cases tree memory was filled before the shortest trees were found. In particular, for Analysis II, some runs terminated with trees of length 188 and others with length 187 and for Analysis III some terminated at length 182, others at length 183. The results reported here are for the runs that yielded the greatest number of shortest trees; further analysis may yield yet shorter trees. In PAUP, when a run terminates because of the maximum tree limit, any non-minimal trees in memory (awaiting branch-swapping) are automatically discarded, so the number of most parsimonious trees found may be less 32,700. Search histories, which indicate discarded trees, are given in the figure captions of the strict consensus trees.

Analysis I

Reanalysis of the original data matrix yielded 3,149 trees of length 193. *Benthobia*, one of the designated outgroups, fell in the ingroup in 100% of the 3,149 trees (as seen from the partition list generated by PAUP), although its position was unresolved in the strict consensus tree (Fig. 2). In the majority rule consensus tree (Fig. 3), *Benthobia* grouped with *Duplicaria* and *Pervicacia* (Pervicaciinae) 82% of the time. Partition analysis showed that *Hastula* (Terebridae) fell as outgroup to all other conoideans in 37% of the trees, and *Conus* was outgroup in 3%. *Conus* plus *Hastula* was outgroup to the other conoideans in 16% (3% as a clade, 13% paraphyletic). Clades that these and the following consensus trees shared with the TKS tree (Fig. 1) are indicated in Table 1.

Analysis II

Reanalysis of the original matrix with *Benthobia* excluded yielded 32,700 trees of length 187. Figs. 4-5 show the strict and majority rule consensus trees. Partition analysis showed that *Conus* did not group within the turrids in 3% of these trees. *Clavus* plus *Splendrillia* fell as outgroup 43% of the time and the three terebrids fell as outgroup 15%. The TKS tree (Fig. 1), which also excluded *Benthobia*, is two steps longer, with length 189.

Analysis III

Reanalysis of the original matrix with *Benthobia* and the hypothetical ancestor excluded yielded 10,490 trees of length 182. Strict and majority rule consensus trees are shown in Figs. 6-7. Of interest here is that the strict consensus tree supports clade 18 of the TKS tree: Conidae including Clathurellinae, Conorbinae, Oenopotinae, Mangeliinae, Daphnellinae, and Taraninae.

DISCUSSION

Although the capability to make data and methods

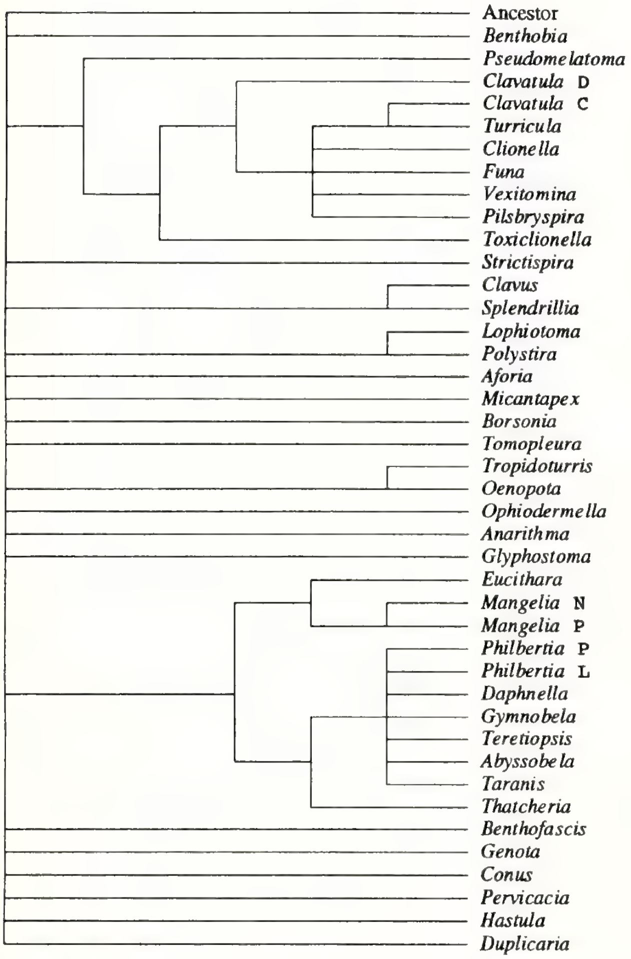
Ancestor
Benthobia
Pseudomelatoma
Clavatula D
Clavatula C
Turricula
Clionella
Funa
Vexitomina
Pilsbryspira
Toxiclionella
Strictispira
Clavus
Splendrillia
Lophiotoma
Polystira
Aforia
Micantapex
Borsonia
Tomopleura
Tropidoturris
Oenopota
Ophiodermella
Anarithma
Glyphostoma
Eucithara
Mangelia N
Mangelia P
Philbertia P
Philbertia L
Daphnella
Gymnobela
Teretiopsis
Abyssobela
Taranis
Thatcheria
Benthofascis
Genota
Conus
Pervicacia
Hastula
Duplicaria

Fig. 2. Analysis I (including *Benthobia*); strict consensus of 3,149 trees, length 193, CI = 0.285, RI = 0.613, HI = 0.715, RC = 0.175. Search history: replicate 1 (seed = 357612083), 1,938 trees found (length = 193); replicate 2 (seed = 1222301227), 395 additional trees found (length = 193); replicate 3 (seed = 63292021), 816 additional trees found (length = 193); replicate 4 (seed = 1306497612), MAXTREES limit (32,700) hit while swapping on tree #11,591, 29,551 non-minimal trees (length = 194) discarded. Total number of rearrangements tried = 407682616, time used = 27:26:26.

explicit is one of the advantages of cladistics over traditional phylogenetic methods, some practitioners of cladistic methods do not take full advantage of this capability. For example Bieler (1990: 371) said of Haszprunar's (1988) "clado-evolutionary" classification of the Gastropoda: "The presentation of the data is incomplete and inconsistent. The analysis is not repeatable" and raised concerns about departure from standard cladistic practices. Reynolds (1997: 20) raised similar issues of "parsimony application and repeatability" in his reanalysis of Steiner's (1992) data set on Scaphopoda. My reanalysis of the conoidean data set further emphasizes that without full details of methods, assumptions, and limitations, results might not be replicable.

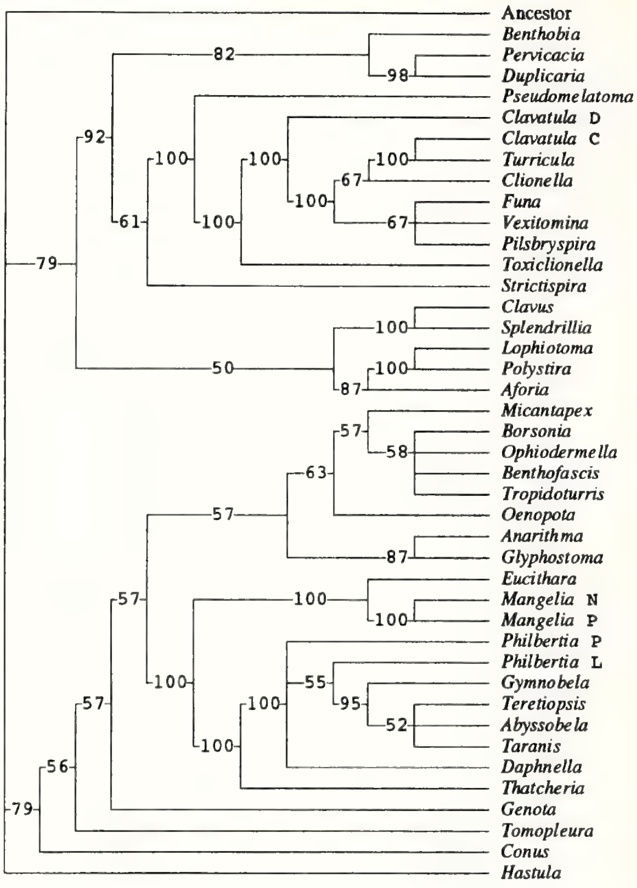
Ancestor
Benthobia
Pervicacia
Duplicaria
Pseudomelatoma
Clavatula D
Clavatula C
Turricula
Clionella
Funa
Vexitomina
Pilsbryspira
Toxiclionella
Strictispira
Clavus
Splendrillia
Lophiotoma
Polystira
Aforia
Micantapex
Borsonia
Ophiodermella
Benthofascis
Tropidoturris
Oenopota
Anarithma
Glyphostoma
Eucithara
Mangelia N
Mangelia P
Philbertia P
Philbertia L
Gymnobela
Teretiopsis
Abyssobela
Taranis
Daphnella
Thatcheria
Genota
Tomopleura
Conus
Hastula
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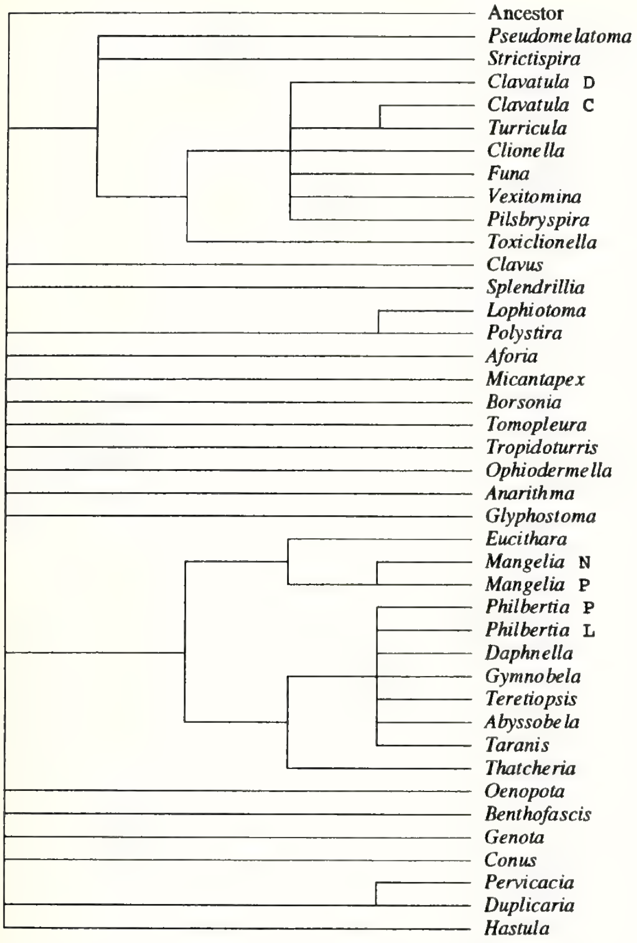
Fig. 3. Analysis I (including *Benthobia*); 50% majority-rule consensus of 3,149 trees of length 193.

I will show first that my Analysis II used the same data and the same assumptions as the analysis presented by Taylor *et al.* (1993), but got different results because of differences in computing power. I will then consider how different assumptions about outgroups, characters, taxa and classification might affect the reliability of the results.

RECONSTRUCTING THE METHODS

Data matrix and ordering of characters

From their stated methods, it is not clear whether Taylor *et al.* intended all characters to be unordered. On page 152, they flagged 18 characters as unordered: (2, 7, 23, 25-31, 33-38, 41-42), but in their table 2, only 15 characters were marked as unordered (2, 7, 23, 26-28, 30-31, 34-38, 40-41). Discrepancies involve characters 25, 29, 33, 40, and 42. All multistate characters were included on one list or the other, so presumably the published analysis was run with all characters unordered. This assumption is supported in that few of the multistate characters are appropriate for linear orderings, but no alternate orderings were suggested. Oddly, a few binary characters (28, 30, 33, 35-36,


Ancestor
Pseudomelatoma
Strictispira
Clavatula D
Clavatula C
Turricula
Clionella
Funa
Vexitomina
Pilsbryspira
Toxiclionella
Clavus
Splendrillia
Lophiotoma
Polystira
Aforia
Micantapex
Borsonia
Tomopleura
Tropidoturris
Ophiodermella
Anarithma
Glyphostoma
Eucithara
Mangelia N
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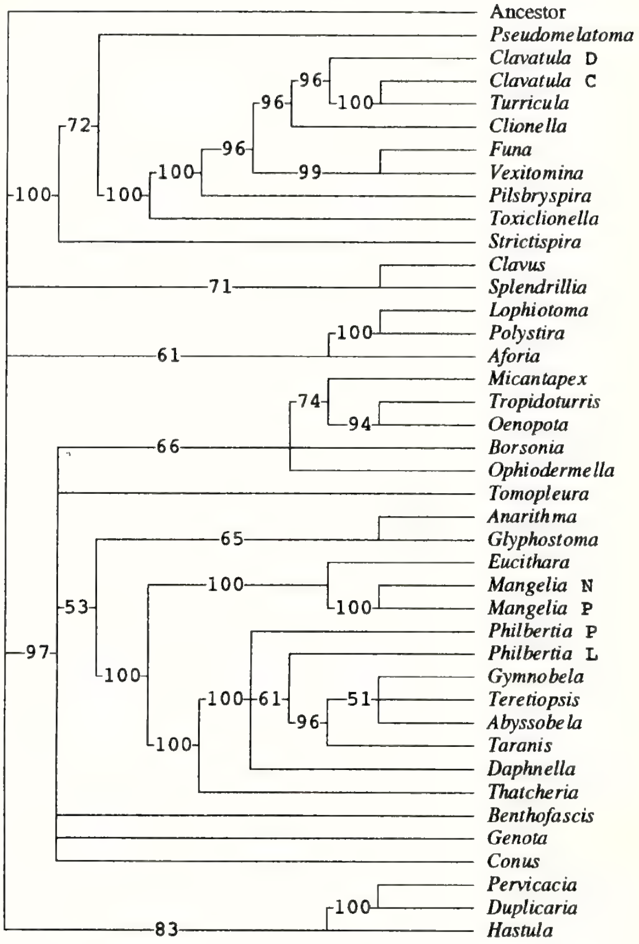
Philbertia P
Philbertia L
Daphnella
Gymnobela
Teretiopsis
Abyssobela
Taranis
Thatcheria
Oenopota
Benthofascis
Genota
Conus
Pervicacia
Duplicaria
Hastula

Fig. 4. Analysis II (excluding *Benthobia*); strict consensus of 32,700 trees, length 187, CI = 0.294, RI = 0.617, HI = 0.706, RC = 0.182. Search history: replicate 1 (seed = 581470058), 31,351 trees found (length = 187); replicate 2 (seed = 426658209), MAXTREES limit (32,700) hit while swapping on tree #31538, 1,349 additional trees found (length = 187). Total number of rearrangements tried = 764211632, time used = 24:40:51.


Ancestor
Pseudomelatoma
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Clavatula C
Turricula
Clionella
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Pilsbryspira
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Strictispira
Clavus
Splendrillia
Lophiotoma
Polystira
Aforia
Micantapex
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Oenopota
Borsonia
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Tomopleura
Anarithma
Glyphostoma
Eucithara
Mangelia N
Mangelia P
Philbertia P
Philbertia L
Gymnobela
Teretiopsis
Abyssobela
Taranis
Daphnella
Thatcheria
Benthofascis
Genota
Conus
Pervicacia
Duplicaria
Hastula
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72
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99
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100
100
71
100
61
74
94
66
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100
100
53
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100
100
61
51
96
100
100
83

Fig. 5. Analysis II (excluding *Benthobia*); 50% majority-rule consensus of 32,700 trees of length 187.

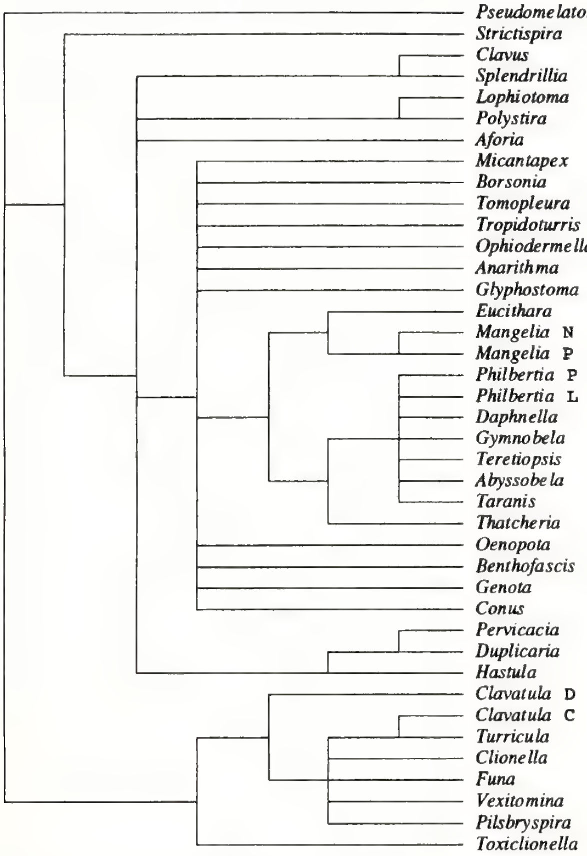
41-42), which are by definition unordered, appeared on each list.

The discrepancies in the lists of unordered characters raise the possibility that through some error the published matrix is not the version of the matrix that was analyzed to produce the TKS tree. Taylor *et al.* (1993: 152) referred to making "small adjustments to the data set" as they explored their data, and Taylor and Kantor (pers. comm.) stated that they tried various scorings of characters and experimented with both ordered and unordered characters. Assuming that all the characters were unordered (and equally weighted), I used MacClade to determine the length of the TKS tree, which, although it is a consensus tree, is fully resolved. It does indeed have the stated length of 189 steps. Thus the published matrix is consistent with the published tree, and with the assumption that all characters were unordered.

Exclusion of the outgroup

Analysis I (original data matrix) found 3,149 trees of length 193 (Fig. 2-3). Taylor *et al.* (1993: 152) reported "over 900" trees of length 189, four steps shorter, for this matrix. Their consensus tree (Fig. 1), however, omits *Benthobia*, which was selected as the outgroup being "the most primitive non-conoidean neogastropod known" (1993: 152). In all trees of length 193, *Benthobia* fell within the ingroup, usually grouping with the Pervicaciinae. Taylor and Kantor (pers. comm.) stated that they excluded *Benthobia* from the analysis because of this behavior. Thus the analysis presented by Taylor *et al.* corresponds to Analysis II herein.

I tried forcing *Benthobia* to stay in outgroup position, using the topological constraints option of PAUP. A heuristic search as described above resulted in 32,700 trees


Pseudomelatoma
Strictispira
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Lophiotoma
Polystira
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Tropidoturris
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Glyphostoma
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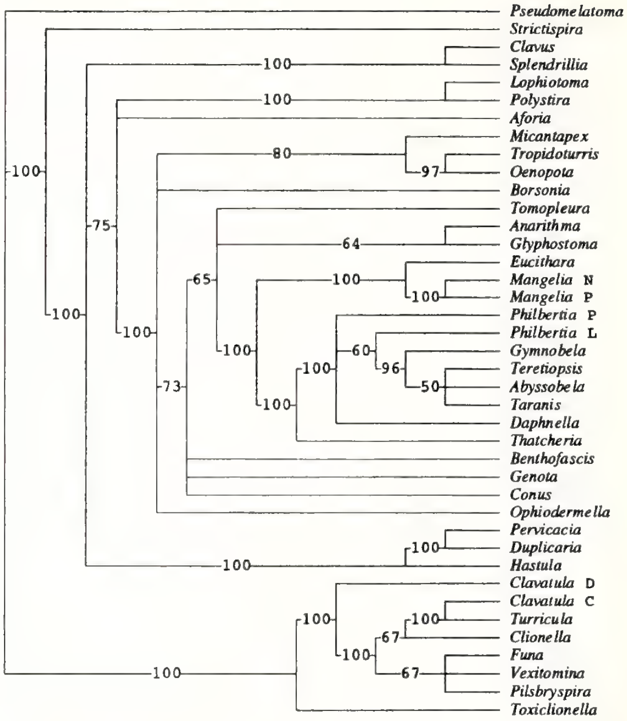
Genota
Conus
Pervicacia
Duplicaria
Hastula
Clavatula D
Clavatula C
Turricula
Clionella
Funa
Vexitomina
Pilsbryspira
Toxiclionella

Fig. 6. Analysis III (excluding *Benthobia* and hypothetical ancestor); strict consensus of 10,490 trees, length 182, CI = 0.302, RI = 0.622, HI = 0.698, RC = 0.188. Search history: replicate 1 (seed = 1): 10,490 trees found (length = 182); replicate 2 (seed = 1459270432): MAXTREES limit (32,700) hit while swapping on tree #16,743, 22,210 non-minimal trees (length = 183) discarded. Total number of rearrangements tried = 403556096, time used = 16:25:50.


Pseudomelatoma
Strictispira
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Tropidoturris
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Glyphostoma
Eucithara
Mangelia N
Mangelia P
Philbertia P
Philbertia L
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Pervicacia
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Toxiclionella
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100
100
80
97
75
64
65
100
100
100
100
100
100
60
96
50
73
100
100
100
100
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67
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67
100

Fig. 7. Analysis III (excluding *Benthobia* and hypothetical ancestor); unrooted, 50% majority-rule consensus of 10,490 trees of length 182.

of length 194, one step longer than without the constraint. The strict consensus and majority rule consensus trees were quite similar to those for Analysis III (Figs. 6-7), in which the outgroups were excluded, but slightly more resolved because of small differences in support for a few clades (*e. g.* 100% versus 96%, 53% versus 47%) and so are not shown here.

Shortest trees

Analysis II (with *Benthobia* excluded) yielded 32,700 trees of length 187, two steps shorter than those found by Taylor *et al.* I ran the matrix several times using different starting seeds. In my analyses, runs always terminated because PAUP's maximum limit of 32,700 trees was reached. This generally took ca. 24 hr. In some cases, trees of length 187 were found in only a few minutes; in others, the shortest trees found before memory overflowed were length 188. In no replicates were trees of length 189 retained as the shortest trees. Taylor and Kantor (pers. comm.) told me that their runs terminated when the computer they used ran out of memory. This explains why Taylor *et al.* found trees of length 189 rather than 187 or 188 and why they found only 900+ trees rather than many thousands of trees: they simply did not have enough computing power. This should have been stated in the article, however, which otherwise gives the impression that the heuristic search routine was completed. Because Taylor *et al.* did not state the starting conditions of their analysis (*e. g.* MAXTREES or other search settings) or the ending conditions, their results could not be reproduced.

HYPOTHETICAL ANCESTOR

Because there is no obvious sister group for Conoidea, Taylor *et al.* (1993: 152) used a hypothetical ancestral taxon "consisting of the underived states, where known, of the characters used in the analysis." Construction of such a hypothetical ancestor is not unusual. A common procedure is to consider a variety of potential outgroups. If only one state occurs in the possible outgroups, the polarity is clear (Donoghue and Cantino, 1984). Taylor *et al.* apparently applied this type of reasoning in some cases (*e. g.* for accessory salivary glands; 1993: 142), but in many cases did not state how they made decisions about polarity. In other cases, the reasons they gave are known to be invalid,

Table 1. Consensus of consensus trees: clades co-occurring in each of the six consensus trees (Figs. 2-7) and the TKS tree (Fig. 1). (Trees from Anal[ysis] I, II, III; MRC, majority rule consensus tree; SC, strict consensus tree).

	Clade shared by tree in:					
TKS Clade	Fig. 2 Anal I SC	Fig. 4 Anal II SC	Fig. 6 Anal III SC	Fig. 3 Anal I MRC	Fig. 5 Anal II MRC	Fig. 7 Anal III MRC
1	x	-	x	x	x	x
2	-	-	-	-	-	-
3	-	-	x	-	x	x
4	-	x	x	x	x	x
5	-	-	-	-	-	-
6	-	x	-	x	x	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	x	x	x	x	x	x
10	x	-	x	x	-	x
11	-	-	-	x	-	x
12	x	x	x	x	x	x
13	-	-	-	x	-	x
14	-	-	-	-	-	-
15	-	-	-	-	-	x
16	-	-	-	x	x	-
17	x	x	x	x	x	x
18	-	-	x	x	x	x
19	-	-	-	-	-	-
20	-	-	-	-	-	-
21	-	-	-	-	-	-
22	-	-	-	-	-	-
23	-	-	-	-	-	-
24	-	-	-	x	x	x
25	-	-	-	-	-	-
26	-	-	-	-	-	-
27	-	-	-	-	-	-
28	-	-	-	-	-	-
29	x	x	x	x	x	x
30	x	x	x	x	x	x
31	x	x	x	x	x	x
32	x	x	x	x	x	x
33	x	x	x	x	x	x
34	-	-	-	-	-	-
35	-	-	-	-	-	-
36	-	-	-	x	x	x
37	-	-	-	x	-	x
38	-	-	-	-	-	-
Total shared clades	10	10	13	19	16	19

such as the three criteria they explicitly mentioned (1993: 132). These are occurrence of character states (1) in taxa with the least derived states of other characters, (2) in most of the subfamilies of Turridae (*i. e.* common equals primitive), and (3) in distant rather than immediate outgroups (some members of Archaeogastropoda or "Mesogastropoda" rather than other Neogastropoda).

The first criterion assumes that primitive character states are correlated, but as Stevens (1980: 334) noted, "Correlations do not necessarily indicate anything about the evolutionary polarity of the characters states involved ... character states may be associated in various ways: all primitive, all derived, or mixed ..." Of the second criterion, Stevens (1980: 335) stated, "the frequency of any character state in a group depends on the subsequent evolutionary history of the lineage with that state and is independent of the evolutionary polarity of that state." Thus a primitive character might be rare if it belongs to a lineage that has not diversified or has suffered much extinction. A derived character might be common if it arose early in the history of a clade. The third criterion ignores that the character states of distant outgroups cannot override the states in closer outgroups; at best they can render polarity ambiguous with regard to the ingroup (Maddison *et al.*, 1984: fig. 3). Also, at least in some cases, Taylor *et al.* applied the first two criteria within the ingroup, rather than doing outgroup comparisons (*e. g.* buccal mass position; 1993: 132).

Because of doubt about the scoring of character states for the hypothetical ancestor, I tried running the analysis without it (and without *Benthobia*) (Analysis III), finding 10,490 trees of length 182. Curiously, the consensus trees for these (Figs. 6-7) have more clades in common with the TKS tree than the consensus trees when the hypothetical ancestor was included: 13 versus ten (strict); 19 versus 16 (majority rule) (Table 1). In particular, clade 18, which groups Conidae with a subset of Turridae, appears in the strict consensus.

CONSENSUS OF THE CONSENSUS TREES

The strict consensus trees (with and without the outgroups) each share only ten to 13 clades with the TKS tree (Table 1), which has a total of 38 clades. Only eight clades are shared by all three strict consensus trees and the TKS tree (node numbers in parentheses): Clavatulinae (part) + Crassispirinae + Zonulispirinae (9); *Clavatula caerulea* + *Turricula* (12); Turrinae (17); Mangeliinae + Daphnellinae + Taraninae (29); Mangeliinae (30); *Mangelia* (31); Daphnellinae + Taraninae (32); and *Philbertia* + *Daphnella* + *Gymnobela* + *Teretiopsis* + *Abyssobela* + *Taranis* (33). One clade appears in all three strict consensus trees that does not appear in the TKS tree: Clavatulinae (all) + Crassispirinae + Zonulispirinae.

Only 13 of the 38 clades are shared by all three majority-rule consensus trees, those in the strict consensus trees, listed above, and: Drilliidae (1); Pervicaciinae (4); Conidae *sensu* Taylor *et al.* (18); *Anarithma* + *Glyphostoma* (24); and *Gymnobela* + *Teretiopsis* + *Abyssobela* + *Taranis* (36). Seventeen clades supported by the Taylor *et al.* analysis are not supported by any of the trees presented herein: 2, 5, 7, 8, 14, 19, 20, 21, 22, 23, 25, 26, 27, 28, 34, 35, and 38.

Taylor *et al.* (1993: 152) did not state the level of support for any of the nodes in their consensus tree, saying instead "most of the branches are supported in 75-100% of the trees." Because of this omission, it is not possible to tell where disagreement is significant. Lack of support in my analysis for a node supported in only 55% of their trees would not be surprising; lack of support for a node supported in 100% of their trees would be more interesting. The 17 nodes contradicted by my analysis average only 1.8 characters per node (Taylor *et al.*, 1993: table 4), whereas the 12 nodes supported by all three of my consensus trees average 3.3 characters per node. The mapping of characters to nodes is often ambiguous, however, and many alternative mappings to the ones in their table 4 exist. For example, character 3(1) was mapped to node 3, with loss in *Pervicacia*. An alternate mapping is independent gain in *Hastula* and *Duplicaria*.

CHARACTERS

Character mappings

In addition to some characters in table 4 of Taylor *et al.* having alternate mappings, some mappings are simply incorrect. Kohn and McLean (1994) noted that character state 39(0) at node 27 (*Benthofascis* + *Conus*) should be mapped at node 26 instead, because it is present in *Genota*. Actually, 39(0) is a primitive state inherited from the root of the tree. State 39(1) (teeth on the outer lip) evolved at nodes 24 and 33 and in *Eucithara*; it was lost at node 36. There is no need to map 39(0) elsewhere in the tree. Similarly, character states 20(1), 35(0), and 38(1), mapped to node 1 (Drilliidae), are actually basal to the conoideans. Also at node 1, *Clavus* has state 7(2) not 7(1). Thus four of the five synapomorphies attributed to Drilliidae in Taylor *et al.'s* table 4 do not map to that node (in the given topology). The remaining character state, 37(0), has an ambiguous mapping, as node 3 has the same state, which thus could be basal, changing to 37(1) at node 5.

Parallelism

The majority of characters (77%) that Taylor *et al.* used are from a single organ complex, the foregut. Any classification based primarily on characters from one system is likely to be mislead by parallelisms. Cladograms based on characters from many organ systems are less likely to be misleading (Davis, 1979: 9). Taylor *et al.* (1993: 151) noted:

> many of the morphological trends in the Terebrinae involve partial to total loss of the foregut organs ...thus many of the characters were recorded as missing. In our earlier attempts at cladistic analysis, terebrid species tended to appear in rather disparate positions on the cladograms. Consequently, we have used only three species to represent the Terebrinae and Pervicaciinae, the taxa being the least-derived known for each group.

Inclusion of characters from the reproductive, circulatory, excretory, and respiratory systems might have overridden the problem caused by the supposed parallel losses in the Terebridae. Paradoxically, understanding of the evolution of the foregut organs requires study of the other organ systems - they cannot be studied in isolation. The hope is that homoplasy in any given system will be more than balanced by the overall phylogenetic signal in combined analysis across systems. An excellent example of this is *Benthobia*. Because of homoplasous characters in foregut anatomy, it was pulled into the ingroup, but the overall anatomy and morphology place it securely with the Pseudolividae (Kantor, 1991; see also Bouchet and Warén, 1985). The single species of *Conus* included in the analysis might have been pulled into Turridae because of parallelisms, just as *Benthobia* was pulled into Conoidea.

Choice and scoring of characters

Some of the ten non-foregut characters analyzed were overly simplified. For example, the division of shell form into "fusiform," "coniform," "turreted," "terebriform," and "rounded" is simply unworkable in the continuum of shapes that occur among the more than 4,000 living species of conoideans, especially because quantitative definitions of these terms were not given (Taylor *et al.,* 1993: 162). Number of protoconch whorls (< 2, > 2) is also an arbitrary division of a continuum, although it tends to correlate with developmental type. Sculpture of the protoconch (absent or weak versus present) combines many disparate types of sculpture as a single character state.

Kohn and McLean (1994) noted that potentially useful shell characters were excluded, such as the resorption of the inner shells whorls found in *Conus* and *Benthofascis*, which Taylor *et al.* mentioned (1993: 155), but did not include in their matrix. Taylor (1994: 435) stated, "We considered, but did not include this character in the cladistic analysis. Its inclusion would have made no difference to the structure of the cladogram except to add another apomorphy at the node of *Benthofascis* and *Conus*." This clade, however, is not supported by any of the consensus trees presented herein. Inclusion of the character might well have resulted in support for this clade.

There are also some inconsistencies in the scoring of foregut characters. Character 15 (septum dividing anterior and posterior areas of the rhynchocoel) was listed as present in *Philbertia purpurea, Daphnella, Pervicacia* and *Duplicaria* in Taylor *et al.'s* table 3, but a "probably homologous septum" reported in *Thatcheria* (1993: 129) was not scored in the matrix. For Character 26, *Strictispira paxillus*

(Strictispiridae) has state 1 (Taylor *et al.*, 1993: table 3), which was stated to occur only in Pseudomelatomidae (1993: 136), and *Hastula bacillus* was scored "?" but has state 2 (1993: table 2). For Character 33, *Pseudomelatoma, Splendrillia, Toxiclionella,* and *Lophiotoma* were scored as having unfused odontophoral cartilages in table 3, but stated to have fused cartilages on p. 133.

CHOICE OF TAXA

Although one of the major conclusions of the study was that some subfamilies of Turridae should be transferred to Conidae, only a single species of *Conus* from among the more than 500 living species was included in the analysis (two species were studied [Taylor *et al.*, 1993: table 1]). The study thus did not provide evidence that *Conus* is monophyletic. Similarly, six of the subfamilies included in the analysis were represented by only one species each. Again, no evidence was provided that these groups are monophyletic, yet two, Pseudomelatomidae and Strictispiridae, were elevated to familial rank. Other taxa studied were excluded from the cladistic analysis, in the case of some Terebridae explicitly because they did not group as expected. Many other species listed in Taylor *et al.'s* table 1 did not appear in their table 3, but no explanation was given for their exclusion. One hopes that they were excluded because of insufficient data, or because character scores did not differ from those of included species, rather than for cladistic misbehavior.

CLASSIFICATION

The classification that Taylor *et al.* introduced does not follow the structure of their cladogram (Fig. 1), as noted by Kohn and McLean (1994): Turridae, Clavatulinae, and Clathurellinae are polyphyletic, and Crassispirinae, Conorbinae, and Daphnellinae are paraphyletic. Taylor *et al.* stated (1993: 157), "Information from taxa not included in the cladistic analysis (mainly radular characters) has also been used in constructing the classification." Presumably these taxa were not included because other data on their foregut anatomy was not available. Unfortunately, inclusion of such information, however relevant, adds another layer of irreproducibility to the analysis. Such data might be used to chose between equally parsimonious trees, but beyond that, there seems little justification for their use.

Taylor *et al.*'s classification conflicts with their tree because it is in part conservative. An alternative classification more consistent with the tree would introduce new subfamily names for parts of Clavatulinae, Clathurellinae, and Crassispirinae, and synonymize Conorbinae with Coninae, and Taraninae with Daphnellinae. That Taylor *et al.* refrained from naming new taxa is appropriate, given the preliminary nature of the data. This conservatism is at odds, however, with the elevation of Strictispiridae and Pseudomelatomidae to family level, which renders Turridae polyphyletic instead of paraphyletic.

The essential conflict of the tree is that Conidae falls within Turridae. The solution that Taylor *et al.* adopted (transferring some turrid subfamilies to Conidae) left Turridae paraphyletic (ignoring for the moment the rank of Strictispiridae and Pseudomelatomidae). This is no better than accepting the traditional classification, in which Turridae might also be paraphyletic. At several points Taylor *et al.* stressed the limitations of their analysis. They noted that "small adjustments to the data set produced rather large changes in tree topography [*sic*] and the number of alternative trees generated" (1993: 152), uncertainty about outgroups (p. 152), and inadequate sampling of taxa (p. 150). In view of these uncertainties, it is not clear why they introduced a new classification - producing the data and cladogram would have been enough.

CONCLUSIONS

The results of Taylor *et al.* (1993) cannot be reproduced from their stated methods. The trees they reported are not minimal trees because the analysis they performed terminated prematurely, being limited by memory of their computer. Further problems arise from omission of the stated outgroup, *Benthobia*, from the cladistic analysis, incorrect character mappings, and conflicts between text and data matrix. The classification that Taylor *et al.* introduced does not follow the structure of their cladogram, taking into account data from taxa not included in the cladistic analysis. This adds further layers of irreproducibility to the results.

Taking the data matrix as given, fewer than half of the clades they found were supported by my reanalysis. The matrix, however, is probably not a reliable basis for a phylogenetic analysis. Most of the characters used were from a single organ complex, the foregut, making it likely that the parsimony analysis was mislead by parallelism, as evidenced by the pseudolivid outgroup, *Benthobia*, being pulled into the ingroup. Also, some of character states of the hypothetical ancestor were determined by unreliable methods such ingroup comparison, so the polarities of characters were not well established. Addition of outgroup and ingroup taxa, rescoring of some characters, and of the hypothetical ancestor, and addition of characters from other organ systems would undoubtedly further modify the results.

Nonetheless, I did find support for some aspects of the Taylor *et al.* analysis, taking the data matrix as given. Mangeliinae + Daphnellinae + Taraninae (node 29), and subsets thereof are supported in the strict consensus trees, as is Turrinae, and Clavatulinae + Crassispirinae +

Zonulispirinae (9), with *Toxiclionella* pulled in also, making Clavatulinae paraphyletic rather than diphyletic. Most interesting is that node 18, Conidae *sensu* Taylor *et al.* (Conidae + Clathurellinae, Conorbinae, Oenopotinae, Mangeliinae, Daphnellinae, and Taraninae) appears in all three majority-rule consensus trees. Support for this clade is not unequivocal, however, as it does not appear in two of the strict consensus trees. Given the limitations of the character data and taxon sampling, it is yet possible that Conidae and Turridae will prove to be two separate clades. The single *Conus* species included in the analysis could well have been pulled into Turridae because of parallelisms, just as *Benthobia* was pulled into Conoidea. Malacologists will be justified in retaining the traditional classification, until more robust evidence for transferring turrid subfamilies to Conidae and for elevating Drilliidae, Pseudomelatomidae, and Strictispiridae to familial status is produced.

Given the authors' cautions about the preliminary nature of their analysis, their introduction of a new classification was premature. The monumental achievement of presenting the anatomical descriptions, assembling the data matrix, and presenting a preliminary tree would have been sufficient. Certainly it is much better to have this data available now than to wait five or ten years while the authors tackled other taxa and other organ systems to produce a better-supported phylogeny. One advantage of cladistic over traditional methods is that intermediate results can be presented without the need to introduce a new classification. Once a new classification is introduced, inevitably some will adopt it, even if it is likely soon to be superseded. For example Turgeon *et al.* (1998) adopted Taylor *et al.'s* new classification of Conoidea.

I note in closing that it was not the introduction of the new classification itself that spurred this reanalysis but that other workers were adopting a classification that I thought likely to be incorrect. Thus, the new classification did draw attention to ideas that have stimulated scientific discourse. Perhaps it has served its purpose after all.

ACKNOWLEDGMENTS

I thank John D. Taylor (The Natural History Museum, London) and Yuri I. Kantor (A. N. Severtzov Institute of Animal Morphology and Ecology, Moscow) for their willingness to discuss their methods with me. Two anonymous reviewers provided valuable comments on the manuscript.

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AMERICAN MALACOLOGICAL SOCIETY
FINANCIAL REPORT

General Accounts

1997 Income and Expenses

BEGINNING BALANCE (12/31/96)			$113,862.68
INCOME			$46,067.09
Membership Dues (1995, 1996, 1997)		18,159.75	
Membership Dues (1998)		856.00	
Interest and Dividends		2,419.28	
Money Market Account Interest	1,123.40		
Life Membership Endowment Fund	108.92		
Symposium Endowment Funds	1,186.96		
Publications Income		13,428.71	
AMB Foreign Subscriptions	1,824.00		
AMB Domestic Subscriptions	412.44		
AMB Unspecified Subscriptions	2,711.99		
AMB Page Charges	5,243.00		
AMB Reprint Charges	2,985.28		
Sales of Other Publications	252.00		
Annual Meetings		9,782.13	
1991 Meeting Surplus	2,042.09		
1996 Meeting Surplus	7,740.04		
Donations		761.00	
Student Paper Award	500.00		
Symposium Endowment Fund	211.00		
Student Grant Endowment Fund	50.00		
Refunds and Reimbursements		530.22	
Miscellaneous Income		130.00	
EXPENSES			$28,064.51
President Expenses	64.94		
Secretary Expenses	401.30		
Membership Chairman Expenses	67.12		
Treasurer Expenses	988.32		
Affiliate Memberships	275.00		
Banking Fees	35.84		
MC - VISA Fees	448.94		
Incorporation Fee	20.00		
Insurance/Bond Fees	604.00		
Refunds	168.00		
Investment Fees	2.50		
Publication Costs	15,680.96		
AMB	14,002.36		
AMS Newsletter	1,678.60		
Dues Notices	606.00		
Abstract Mailing (1996 Meeting)	1,254.25		
Officers' Travel Expenses (To Annual Meeting)	6,047.34		
Student Paper Awards	1,000.00		
1999 Meeting Deposit	400.00		
NET GAIN IN 1997			$18,002.58
ENDING BALANCE			$126,167.85

65th ANNUAL MEETING
THE AMERICAN MALACOLOGICAL SOCIETY
PITTSBURGH, PENNSYLVANIA, U. S. A.
JULY 4-9, 1999

The 1999 American Malacological Society meeting will be held at the Sheraton Station Square in Pittsburgh, Pennsylvania. In addition to an exciting symposium and various contributed sessions and posters, the meeting will host various special sessions and workshops of unique nature but broad interest.

Symposium: New Looks at Old Molluscs: Recent Perspectives of Molluscan Evolution
Organizers: Bud Rollins, University of Pittsburgh (snail@vms.cis.pitt.edu) and Ellis Yochelson Smithsonian Institution (yochelson.ellis@simnh.si.edu).

Special Sessions and Workshops

–Molluscan Genetics organized by Laura Adamkewicz, George Mason University (ladamkew@gmu.edu).

–Mollusks and Education: New Ideas from Museums to the Classrooms organized by M. Patricia Morse, University of Washington (mpmorse@u.washington.edu).

–Biomineralization in Mollusks (including workshop) organized by Joseph Carter, University of North Carolina (clams@email.unc.edu).

–Women in Malacology organized by Louise Russert-Kraemer, University of Arkansas (rkraemer@comp.uark.edu).

–Malacology Curation Workshop organized and presented by Charlie Sturm, Carnegie Museum of Natural History (csturmjr+@pitt.edu), Gary Rosenberg, Dick Petit, Jose Leal, Tim Pearce, and Kevin Cummings.

–The Clench Tapes and AMU History organized by Harold Murray (hmurray@trinity.edu).

Additional AMS 1999 events will include a July 4th fireworks reception, access to Carnegie Museum of Natural History mollusk collection, auction with special molluscan art available, banquet cruise and a special student reception, plus a keynote address by Rich Lutz, Rutgers University, on "Deep-Sea Hydrothermal Vents: Exciting New Discoveries."

Art Bogan is organizing a field trip to high diversity freshwater sites in western Pennsylvania while Albert Kollar will lead a trip to excellent fossil sites, both on the last day of the meeting.

Pittsburgh is an exciting, compact city with remarkable museums, theaters, and sports. Come early and stay late to take advantage of this completely revitalized city. The venue of the meeting is a full facility conference hotel, the Sheraton Station Square, with ready access to all that Pittsburgh has to offer. Be sure to participate. A call for papers and posters will soon appear; please get your abstracts and registration materials in as early as possible. Deadline is 5 March 1999.

For more information contact:
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IN MEMORIAM

Harold Lewis

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[first occurrence in each paper recorded; new tax (including species) in **bold face**]

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EDITORIAL COMMENT

It is with sadness that I note the recent loss to the science of malacology and to the leadership and membership of the American Malacological Society, of a great friend, loyal devotee, and generous benefactor. Constance E. Boone passed away on September 14 in Australia. It is fitting that she left us while engaged in one of the activities that she loved best, traveling the world, oftentimes well off the beaten path, on collecting trips in search of mollusks. A past president of the American Malacological Society (then the American Malacological Union) and the Houston Conchology Society, Co-Editor of the Texas Conchologist and long-time Associate in the Malacology Department of the Houston Museum of Natural Science, Connie epitomized the knowledgeable and dedicated amateur and helped to strengthen the relationships between and among professional, paraprofessional, and amateur malacologists.

An ardent supporter of undergraduate and graduate students and young professionals in malacology, Connie took great interest in seeing that the profession of malacology was in secure hands for the future. There are many of us who were privileged to witness and many others who were the direct beneficiaries of her many generous contributions to student paper awards and student research in malacology, which helped to ensure the future of her passion and our science and profession.

On a personal level, my memories of two decades of AMS meetings are filled with recollections of Connie's warmth, friendship, and caring. True to her great form and good nature, one of her first questions to me at the most recent AMS meeting this past summer in Pittsburgh was of my wife and three children and how they were adapting to our recent move from Georgia to Arkansas. Her questions then turned to how much time I would have to spend on my research interests and whether I would be able to continue training students in light of my new administrative duties. How typically Connie – thoughtful, supportive, concerned, direct.

It is wonderfully appropriate that at the AMS meeting in Pittsburgh, her last AMS meeting, Connie helped to organize and was a contributing panelist for the session on "Women in Malacology". I was fortunate to have been in the audience that day as she spoke eloquently and passionately about the history of malacology from the perspective of the contributions of those women who were ahead of their time as compared to contemporary standards. In recent years, Connie was particularly pleased to see that women were well represented among the ranks of young professional malacologists and her talk helped to inspire them further to continue in the field and to succeed.

Love and appreciation of friends, family, colleagues, as well as adventure, the discovery of the natural world, and working toward the perpetuation of malacology as an attractive and active hobby and science were the passions that drove her and to which she dedicated much of her life and near boundless energies. Her legacy lives on through the many of us whose lives she touched, shared, and enriched. We will miss her.

Ronald B. Toll
October 1999

The utility of fossil data in phylogenetic analyses: a likelihood example using Ordovician-Silurian species of the Lophospiridae (Gastropoda: Murchisoniina)

Peter J. Wagner

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Abstract: Gastropods have a dense fossil record dating back to the Late Cambrian. Intuitively, this would appear to aid phylogenetic reconstructions. However, workers question both whether gastropod shell characters are phylogenetically informative and whether stratigraphic data can be used to test phylogenetic hypotheses. Both questions are addressed with an analysis of 82 species of the Lophospiroidea (= Lophospiridae + Trochonematidae) from the Ordovician and Silurian. Compatibility analyses of 95 shell characters shows that characters are far more compatible than one would expect from homoplasy-saturated data. However, compatibility among the 73 characters that vary among the earliest lophospirids decreases over time, which suggests that later species introduced homoplasy. Simulations using 95 characters and sampling similar to that observed for lophospirids show that parsimony performs poorly relative to methods that incorporate stratigraphic data such as stratocladistics. An alternative approach is used here. The first step is to estimate the likelihood of a hypothesized tree given observed character congruence (*i. e.* parsimony length) using simulations. The second step uses two different statistical tests to estimate the likelihood of hypothesized trees given observed stratigraphic data. Likelihoods then are combined to evaluate trees. The resulting likelihood tree is nearly 30 steps longer (378.4 versus 350.5), but is considered more likely (given a 350.5-step matrix) than a tree of 350.5 steps. Both trees suggest that budding cladogenesis (where ancestors co-exist with descendants) was the most common pattern of speciation, although the likelihood tree is more emphatic on this point. Both suggest a trend towards increasing numbers of ornate species; however, whereas parsimony suggests the differential diversification of an ornate clade, likelihood suggests a strong tendency for inornate ancestors to have ornate descendants. A genus-level taxonomic revision is provided that is consistent with both trees and that attempts to reflect historical diversity patterns.

Key words: phylogeny, likelihood, fossils, Paleozoic, gastropods

Gastropods have a rich fossil record coupled with extensive ecologic and morphologic diversity among extant species. These factors make the clade an excellent model for testing macroevolutionary hypotheses (Bieler, 1992). Most macroevolutionary hypotheses make predictions about phylogenetic patterns. Therefore, robust phylogenetic estimates offer means of testing alternative macroevolutionary hypotheses. Examples of such hypotheses that have been tested in a phylogenetic context with fossil gastropod data include hypotheses about speciation patterns (Wagner and Erwin, 1995), predator-prey escalation (Carlson and Vermeij, 1996), phylogenetic constraints (Wagner, 1995a), and long-term trends (Wagner, 1996).

Unfortunately, fossilized character data for gastropods are limited almost entirely to shell characters, which likely are highly homoplastic (*e. g.* Harasewych, 1984; Kool, 1993). Phylogenetic methods such as parsimony likely will perform poorly with shell data because much character congruence will reflect convergence rather than shared ancestry (Felsenstein, 1984; Archie, 1996). The dense fossil record of gastropods offers a potential antidote to the homoplasy problem because phylogenetic hypotheses make necessary predictions about durations, which in turn make probabilistic predictions about stratigraphic ranges. Accordingly, several workers have used stratigraphic data to test whether characters linking taxa are better explained as convergences than as homologies (*e. g.* Fisher, 1991, 1994; Huelsenbeck, 1994; Cheetham and Jackson, 1995; Wagner, 1995b).

This paper will attempt to address whether shell character data retain phylogenetic signal and whether incorporating stratigraphic data improves estimates of phylogenies. Shell character and stratigraphic data for Ordovician and Silurian members of the Lophospiroidea (= Lophospiridae + Trochonematidae) are analyzed using compatibility, parsimony, and maximum likelihood techniques to contrast alternative hypotheses about lophospiroid relationships and character evolution. The implications of the different results for macroevolutionary hypotheses and lophospiroid classification also are discussed.

This paper is a contribution to the 1997 AMU Symposium on Traditional Versus Phylogenetic Systematics of Mollusks. See *American Malacological Bulletin* 14(2):189.

DATA AND ANALYSES

SHELL CHARACTER DATA, ANALYZED SPECIES, AND PARSIMONY

A previous phylogenetic analysis of lophospiroids used 57 characters and 150 character states to estimate relationships among 55 Ordovician species (Wagner, 1995b). The analysis presented here uses 95 characters encompassing 257 character states for 82 Ordovician and Silurian species. The increased number of character states reflects in part the addition of Silurian species with characters and character states unobserved among Ordovician species. Also, the characters were re-coded following Wagner [in press (a)]. Many characters from the 1995 analysis are divided into two or more characters that better reflect variations observed among Early Paleozoic gastropods. Nevertheless, 95 characters might appear to be a large number for a group of gastropods that previously were divided into only two families. However, a survey of numerous phylogenetic studies of gastropods shows that the ratio of shell characters to analyzed taxa increases as the taxonomic level of the analysis becomes finer [Wagner, in press (a)]. Also, lophospiroids are a morphologically diverse clade. Many more characters are needed to described this diversity than are needed to describe any one species.

The characters are listed and described in Appendix 1. The character matrix is given in Appendix 2. Continuous characters are divided into ordered series using segment coding and then de-weighted so that the maximum difference equaled one step (Chappill, 1989). As a result, alternative trees often have fractional lengths. Other multistate characters are treated as ordered if they represented a logical geometric series; otherwise, they are considered unordered. Some characters, such as the shapes and dimensions of the right and left ramps, vary both in symmetry (*i. e.* left and right being nearly identical) and dimensions. For such characters, symmetry versus asymmetry is coded as one character. Left and right characters are coded separately if they varied independently among asymmetric species. However, left and right characters necessarily covary on symmetric species, which leads to a conundrum: devising a coding scheme where the difference in left and right ramp shape accounts for one difference between two symmetrical species, two differences between two asymmetrical species, and two or three differences between a symmetrical and an asymmetrical species (one difference reflecting symmetry:asymmetry, with the third difference apparent only if both the left and right ramps differ). Step matrices (Swofford and Olsen, 1990) offer a potential solution, but these result in exorbitant run times. Instead, asymmetrical characters are weighted one-half of presence/absence characters, making changes among symmetrical species equal to one step. Trees are rescaled using a separate program that insures no "chimera" reconstructions (*e. g.* hypothesized symmetrical ancestors with different left and right sides), with the tree lengths now scaled as they would be by a step matrix.

Broader phylogenetic analyses [Wagner, in press (a)] suggest that lophospiroids are nested within the Murchisoniina, with species assigned to the genus *Ectomaria* representing the immediate outgroup. Therefore, *Ectomaria adelina*, which is both the earliest known *Ectomaria* and contemporaneous with the oldest lophospiroids, is included as an outgroup. Another contemporaneous murchisoniinae species, *Hormotoma simulatrix*, also is included as an outgroup.

Heuristic parsimony analyses using PAUP 4.0* (Swofford, 1998) found 36 trees of 350.8 steps. The illustrated tree (Fig. 1) is the parsimony tree with the fewest stratigraphic gaps, which satisfies the secondary role for stratigraphy advocated by Smith (1994). [This is equivalent to Clyde and Fisher's (1997) "Analysis 2."] The tree is discussed in greater detail below, but it is presented here to introduce the potential errors for the following sections. The 50 random addition replicates found an additional six islands of trees (Maddison, 1991) with minimum length trees of 380.5 or fewer steps. The phylogenetic reconstruction (Fig. 1) accounts for both stratigraphic ranges and character distributions. Both are important because species identical to reconstructed ancestral morphologies obviate the need for hypothesized range extensions (Fisher, 1991; Smith, 1994).

DO SHELL CHARACTERS CONTAIN PHYLOGENETIC SIGNAL?

Bandel and Geldmacher (1996) criticized a previous phylogenetic analysis of Ordovician lophospiroids by Wagner (1995b) because the resultant phylogenetic estimate linked *Trochonema* and allies to lophospirids. Bandel and Geldmacher (1996) considered these to be distant relatives based on Triassic specimens. Those authors attributed Wagner's (1995b) results to pervasive homoplasy among shell characters. There is some support for Bandel and Geldmacher's (1996; hereafter B&G) hypothesis. Parsimony reconstructions based on soft anatomy suggest high frequencies of homoplasy among shell characters (*e. g.* Carlson and Vermeij, 1996; Haasl, 1997). The utility of shell characters (on which this analysis necessarily relies) must be questioned and the B&G hypothesis must be tested before proceeding with further analyses.

Hypothesized phylogenies predict hierarchical structure among homologies. Conversely, a hypothesis of pervasive homoplasy (such as the B&G hypothesis) predicts little hierarchical signal among the characters. However, the B&G hypothesis does not predict an absence of congruence. Although the terms often are confounded

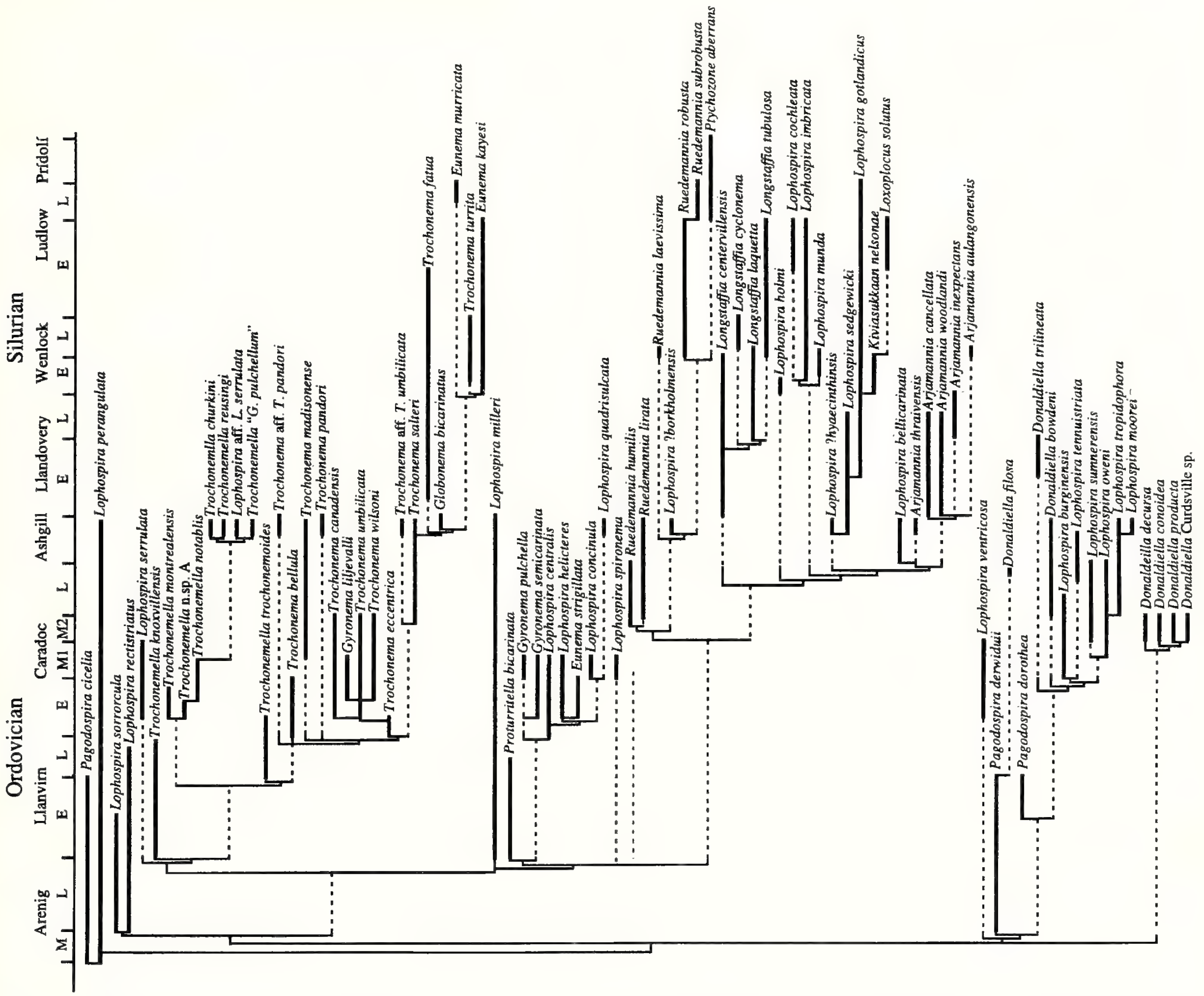

Ordovician
Silurian
Arenig
Llanvirn
Caradoc
Ashgill
Llandovery
Wenlock
Ludlow
Pridolí
M
L
E
L
E
M1
M2
L
E
L
E
L
E
L
Pagodospira cicelia
Lophospira perangulata
Lophospira sorrorcula
Lophospira rectistriatus
Lophospira serrulata
Trochonemella knoxvillensis
Trochonemella montrealensis
Trochonemella n.sp. A
Trochonemella notablis
Trochonemlla churkini
Trochonemella reusingi
Lophospira aff. L. serrulata
Trochonemella "G. pulchellum"
Trochonemella trochonemoides
Trochonema aff. T. pandori
Trochonema bellula
Trochonema madisonense
Trochonema pandori
Trochonema canadensis
Gyronema liljevalli
Trochonema umbilicata
Trochonema wilsoni
Trochonema eccentrica
Trochonema aff. T. umbilicata
Trochonema salteri
Trochonema fatua
Globonema bicarinatus
Eunema murricata
Trochonema turrita
Eunema kayesi
Lophospira milleri
Proturritella bicarinata
Gyronema pulchella
Gyronema semicarinata
Lophospira centralis
Lophospira helicteres
Eunema strigillata
Lophospira concinula
Lophospira quadrisulcata
Lophospira spironema
Ruedemannia humilis
Ruedemannia lirata
Ruedemannia laevissima
Lophospira ?borkholmensis
Ruedemannia robusta
Ruedemannia subrobusta
Ptychozone aberrans
Longstaffia centervillensis
Longstaffia cyclonema
Longstaffia laqueta
Longstaffia tubulosa
Lophospira holmi
Lophospira cochleata
Lophospira imbricata
Lophospira munda
Lophospira ?hyaecinthinsis
Lophospira sedgewicki
Lophospira gotlandicus
Kiviaskkaan nelsonae
Loxoplocus solutus
Lophospira bellicarinata
Arjamannia thraivensis
Arjamannia cancellata
Arjamannia woodlandi
Arjamannia inexpectans
Arjamannia aulangonensis
Lophospira ventricosa
Pagodospira derwidui
Donaldiella filosa
Pagodospira dorothea
Donaldiella trilineata
Donaldiella bowdeni
Lophospira burginensis
Lophospira tennuistriata
Lophospira sumnerensis
Lophospira oweni
Lophospira tropidophora
Lophospira moorei
Donaldiella decursa
Donaldiella conoidea
Donaldiella producta
Donaldiella Curdsville sp.

Fig. 1. Phylogenetic interpretation of one of 36 parsimony cladograms. This tree implies less stratigraphic debt than does any of the other 35 trees. Thin solid lines show estimated phylogenetic connections. Dashed lines show hypothesized unsampled lineages and taxa that are implicit to the phylogenetic hypothesis (*i. e.* range extensions *sensu* Smith, 1988, 1994; = ghost lineages and taxa *sensu* Norell, 1993). The tree posits 350.5 steps [consistency index (CI) = 0.316; retention index (RI) = 0.807) and 102 units of stratigraphic debt, with debt units based on the substages marked on the time scale. Species identical to hypothesized ancestors are linked directly to nodes and are considered ancestral to their apomorphic sister taxa here. [*Trochonemella "G. pulchellum"* is identical to *Trochonemella* BMNH 36364 in Appendix 2.]

(*e. g.* Bryant, 1992; Carpenter, 1992), hierarchy and congruence are not synonyms: congruence exists in non-hierarchical matrices (Källersjö *et al.*, 1992; Alroy, 1994) and even in randomly generated matrices (Archie, 1989; Faith and Cranston, 1991). However, whereas hypotheses of phylogeny and homology predict hierarchical congruence among characters, hypotheses of homoplasy predict non-hierarchical congruence. (The one exception to this expectation is when multicharacter complexes are homoplastic and patterned so that character combinations appear in the same order multiple times; however, this begins to violate another important assumption of phylogenetic methods, *i. e.* the independence of characters.)

Several workers (*e. g.* Meacham, 1984, 1994; Sharkey, 1989, 1994; Alroy, 1994) have suggested using character compatibility (Le Quesne, 1969; Estabrook *et al.*, 1975) to evaluate hierarchical content of matrices. Two binary characters with pairings of (00), (10), (01), and (11) must have homoplasy in one or both characters. Such incompatible characters necessarily lack global hierarchy. Two binary characters with only three of the possible pairs [*e. g.* (00), (10), and (01) or (11)] are considered compatible because they can be reconstructed on some trees without homoplasy. Characters with little homoplasy should form many compatible pairs whereas those with numerous homoplasies should form many incompatible pairs.

Alroy's (1994) Permutation Compatibility (PC) test is operationally similar to the Permutation Tail Probability test (Faith, 1991). PC tests whether the hierarchical structure of a character matrix is significantly greater than that of a randomly produced matrix. Alroy (1994) divided compatibility into two types: hierarchical [= "direct" of Sharkey (1994)] and general ("indirect"). The former describes pairs where combinations show a hierarchical arrangement [*e. g.* (00), (01), and (11)]. The latter describes pairs where there is neither necessary homoplasy nor implied hierarchy [*e. g.* (00), (01), and (10)]. Because phylogenetic hypotheses explicitly predict hierarchy, I used the PC test with 1,000 randomized matrices to determine if randomly permuted matrices ever retained the same level of hierarchical compatibility as did the real matrix. Lophospiroid character data yielded 1,389 hierarchically compatible pairs out of 8,930 possible pairs. (The test has been amended here to compare characters, not character states, as was done in Alroy's implementation). Permuted matrices typically showed approximately half as many pairs (Fig. 2). Thus, the matrix has more hierarchical structure than expected if congruence largely reflected random homoplasy, allowing us to reject the B&G hypothesis.

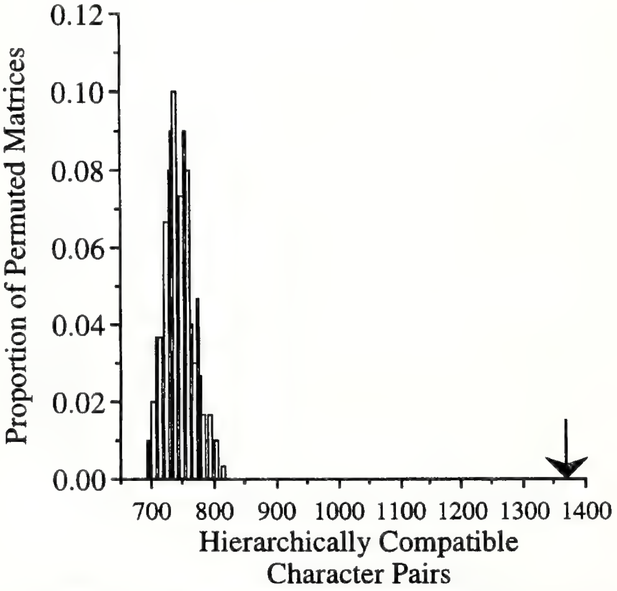


Fig. 2. Results of the Permutation Compatibility test (Alroy, 1994). The arrow denotes the observed value whereas the histogram gives the values generated by 1,000 random permutations of the character data. Hypothesized phylogeny predicts hierarchical compatibility whereas other hypotheses of character congruence (*e. g.* general convergence) do not. Thus, these results support (but do not demonstrate) the idea that there is phylogenetic signal among these characters.

DO WE NEED STRATIGRAPHIC DATA TO IMPROVE OUR ESTIMATES OF LOPHOSPIROID PHYLOGENY?

The presence of some hierarchical structure is no guarantee that there is not sufficient homoplasy to mislead parsimony. Simulation studies show that even low rates of randomly accrued homoplasy will result in inaccurate parsimony reconstructions of phylogeny (*e. g.* Mooers *et al.*, 1995). Finite numbers of character states (see Wagner, 1998c) insure that the probability of chance homoplasies confounding parsimony is not infinitesimal (Felsenstein, 1978). Patterned homoplasies (*e. g.* functional complexes) violate assumptions of character independence and also lead to inaccurate parsimony reconstructions (Lamboy, 1994; Archie, 1996). Finally, rates of character change that are not adequately reflected by character weighting (see Felsenstein, 1981) also yield inaccurate parsimony trees (Kuhner and Felsenstein, 1994).

Neontologists can test phylogenetic hypotheses derived from one data set by examining different character sets (*e. g.* different types of molecules). The hypothesis that congruence from the original data set reflects phylogeny predicts very similar patterns of congruence in other data sets (assuming that changes in one character set do not affect changes in the other; Templeton, 1983). Unfortunately, only a single character set is available for extinct taxa such as lophospiroids. However, phylogenetic hypotheses also make necessary predictions about temporal durations of taxa (see, *e. g.* Smith, 1988). Predictions about durations make probabilistic predictions about stratigraphic ranges (Paul, 1982; Strauss and Sadler, 1989). If a hypothesis makes predictions (necessary or probabilistic) about a data set, then those data offer a test of the hypothesis.

Additional factors suggest that stratigraphic data might improve estimates of lophospiroid phylogeny. Simulation studies [Wagner, in press (b)] show that phylogenetic error exaggerates the phylogenetically implied range extensions (Smith, 1988, 1994; "ghost" lineages and taxa sensu Norell, 1993) far more often than error underestimates range extensions. The exaggeration becomes worse as frequencies of change per character per branch increase, but it is pronounced at the frequencies of change posited by the parsimony tree for lophospiroids (*i. e.* $f = 0.042$ per character per branch). Thus, the long gaps in sampling posited in Fig. 1 are evidence that synapomorphies (sensu Sober, 1988) are homoplastic rather than homologous. For lophospirids, most gaps are highly improbable given observed sampling distributions (Wagner, 1995b).

Another concern is that the hierarchical signal of the character matrix might decrease as geologically younger taxa are added. Suppose that geologically older species have morphotypes (00), (01), and (01), but that later species introduce morphotype (10). The characters no longer are

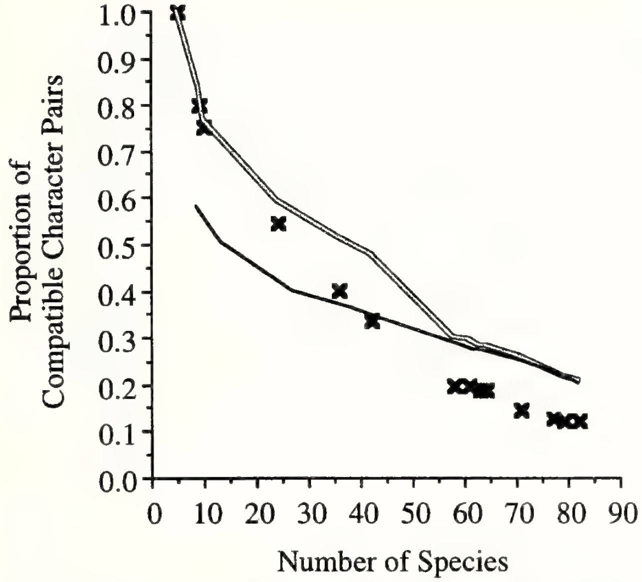


Fig. 3. Effects of geologically younger taxa on overall compatibility. As taxa are added over time, compatibility decreases. The white line gives the compatibility among all characters after X species are added to the matrix. Crosses give the compatibility among only the 73 characters informative for "early" (*i. e.* pre-Caradoc) relations. Thin black line gives the compatibilities for all characters when stratigraphic ranges are assigned at random.

compatible, and the younger species necessarily imply reversal or convergence. Conversely, suppose that the older species had states (00) and (11) and some geologically-younger species had (10). This suggests that the "younger" species is descended from a phylogenetic intermediate that was not sampled initially. The former scenario predicts decreased character compatibility whereas the latter does not.

I examined the effect of geologically younger taxa on character compatibility among lophospirids using all character and also only the 73 characters that vary among early lophospiroid species (Fig. 3). (Late-appearing characters are not relevant to relationships among the oldest taxa.) Compatibility decreases markedly over time. Sampling so poor that species were effectively sampled at random through time relative to their phylogenetic position (as implied by abundant ghost lineages) predicts a very different pattern. One thousand randomizations of stratigraphic ranges show that compatibility should begin lower than observed and that the decrease over time should be much less precipitous (Fig. 3). The observed patterns are consistent with the idea that Late Ordovician and Silurian lophospiroids exhibited homoplasy among the characters that distinguish Early-Middle Ordovician lophospiroids.

A final concern is whether methods incorporating stratigraphic data perform better than does parsimony. ("Parsimony" here and throughout this paper denotes minimum steps evolution [Edwards and Cavalli-Sforza, 1964; Kluge and Farris, 1969]; other methods using parsimony criteria [*e. g.* stratocladistics] are labeled differently.) To test this, I simulated morphologic evolution using the number of characters and states per character apparently available to lophospiroids. Character evolution was ordered or unordered reflecting the assumptions of the phylogenetic analysis (Appendix 1). In one round of analyses, log-probabilities of character change were proportional to the relative weighting of characters (*i. e.* "equiprobable change"; see Felsenstein, 1981). In a second round, rates of change varied among characters at random (*i. e.* "variable change"). Two different speciation models were used, one in which ancestral morphotypes survived cladogenesis and could produce any number of descendants ("budding cladogenesis") and one in which ancestral morphotypes became pseudo-extinct while giving rise to two distinct descendants ("bifurcating cladogenesis"). Sampling parameters derived from lophospiroid data (see Appendix 3) were used to sample six species. (A six-species limit was imposed so that exhaustive searches could be used.) Parsimony analyses then were run using the simulated character matrices. Matrices were maintained only if parsimony estimated the same steps per sampled taxon as the real lophospiroid tree (*i. e.* 350.5 steps per 82 taxa ? 25.5 steps per six taxa; hereafter: a 25.5-step matrix).

The simulations then had character matrices and fossil records that were similar to those observed for lophospiroids. The stratigraphic data then were used for three additional phylogenetic analyses: reweighting with the Stratigraphic Consistency Index (SCI; Huelsenbeck, 1994), stratocladistics (Fisher, 1994), and sieving with 95% confidence intervals (CIS; Wagner, 1995b). The results of the phylogenetic analyses were contrasted using Robinson and Foulds' (1981) metric, which measures the proportion of nodes that agree on two trees. (If multiple optimal trees were found, then the mean error was used.)

All methods incorporating stratigraphic data outperformed parsimony when simulated character matrices and simulated sampling matched observed parameters for lophospiroids (Fig. 4). In the best case, stratocladistics correctly reconstructed over 50% of the cladograms. Parsimony, meanwhile, correctly reconstructed under 15% of the cladograms. Stratigraphic methods are less successful when rates of character evolution are not proportional to character weighting, something that is true of parsimony and other phylogenetic methods (Kuhner and Felsenstein, 1994). However, stratigraphic methods are slightly less affected by such variation than is parsimony.

In summary, lophospiroid character data show too much compatibility to be dismissed as noise. However, reasons to question the parsimony estimate of phylogeny include: (1) many implied but statistically improbable sampling gaps, (2) decreasing hierarchical signal over time, and

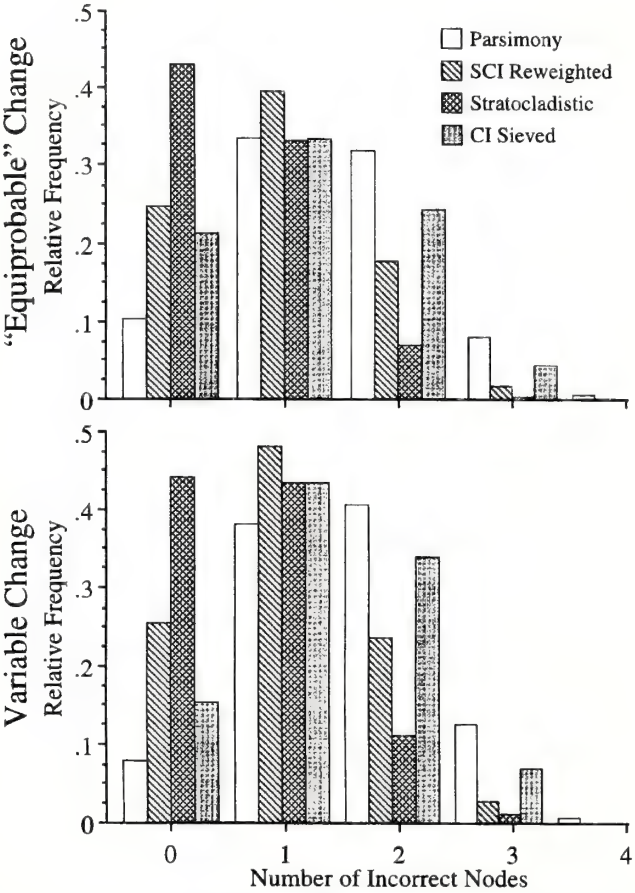


Fig. 4. Success of four phylogenetic methods for six-taxon simulations. The numbers reflect the number of incorrect nodes ("0" indicates a correct tree). The simulations use 95 characters and the same number of possible character states and character state orderings per character as lophospiroid data. Sampling intensities mimic observed sampling. "Equiprobable" evolution used probabilities of character change proportional to the character weight, whereas "variable" evolution used randomly assigned probabilities of change. Results are from budding cladogenesis, in which ancestral species can survive to yield any number of descendants, but the simulations produce nearly identical results when using bifurcating cladogenesis. Note that all three methods using stratigraphic data outperform parsimony. See text for additional discussion.

(3) superior performance of methods incorporating stratigraphic data in simulations.

WHY MAXIMUM LIKELIHOOD APPROACHES ARE USEFUL

How best to incorporate stratigraphic data into phylogenetic analyses is a non-trivial issue. I reject the position that stratigraphic data cannot improve phylogenetic estimates because such data are not hierarchically distributed (*e. g.* Eldredge and Cracraft, 1980; Rieppel and Grande, 1994; Smith, 1994). This only means that stratigraphic data do not offer inductive statements about phylogeny. If the goal is to test the predictions of phylogenetic statements, then one criterion applies: do estimated phylogenies make predictions about stratigraphic data? The syllogism is straightforward: if an inferred phylogeny is accurate, then taxa originated by particular times; if taxa originated by a particular time, then there is a some probability that the known fossil record would be observed. Of course, the "deduction" is necessarily fuzzy, as stratigraphic data cannot demonstrate that a lineage had originated by any point in time, only that it is highly unlikely to have done so given observed data.

Unfortunately, the methods incorporating stratigraphic data used above all are unsatisfactory. Reweighting trees with SCI suffers because the SCI is greatest on pectinate trees (Siddall, 1996). Sieving trees with confidence intervals is not obviously predisposed toward favoring particular topologies. However, CIS suffers from focusing on particular nodes rather than the whole tree (Wagner, 1998b). Multiple independent tests and a traditional significance value of 0.05 should yield Type I errors (*i. e.* incorrectly rejecting the null) one time in every 20 tests. More damning, multiple tests should yield Type II errors (*i. e.* incorrectly accepting the null) even more frequently. For example, if there are ten gaps that are significant at a p-value of 0.10 each, then the CIS method will accept all of them. However, we expect only one of those gaps to be real.

An alternative approach with sounder logical justifications is to use likelihood to evaluate hypothesized trees. Stratocladistics actually represents a simple likelihood test. Summing steps is equivalent to summing negative logs of character change probabilities (Felsenstein, 1981) if: (1) probabilities for each character are the same among all clade members, (2) characters never change twice on the same branch, and (3) characters always evolve independently. The most likely tree then is the one invoking the fewest steps. Summing the negative log-probabilities of implied gaps (*i. e.* stratigraphic debt *sensu* Fisher, 1991, 1994) estimates the log-probability of a tree. Again, the most likely tree is the one implying the fewest stratigraphic gaps. The log-likelihood of the entire tree now equals the sum of stratigraphic debt and morphologic steps *if* the stratigraphic debt is weighted as:

$$-\ln(1 - R) = W * -\ln(P[c])$$

where 1 - R is the probability of not sampling a taxon over a particular interval (Foote and Raup, 1996), W is the weight of stratigraphic debt relative to characters, and P [c] is the probability of character change along a branch.

Stratocladistics will retain nodes implying improbable gaps if they are supported by numerous synapomorphies. This offers a control for Type I errors which is lacking in the confidence interval method. Clyde and Fisher (1997) further justified stratocladistics on the grounds that it uses the same optimality criterion to evaluate all of the data, which is the justification of "total evidence" analyses (*e. g.* Kluge and Wolf, 1993). However, stratocladistics requires *a priori* assumptions about sampling intensity (*R*) and frequencies of character change. As unknowns, both should be tested rather than assumed. Fortunately, the stratigraphic debt for any one tree implies an *R*, the likelihood of which can be assessed analytically (Foote, 1997). *R*'s predicting some debt are more likely than *R*'s predicting no debt (Wagner, 1998a), which means that statistically optimal trees can be "suboptimal" by stratocladistic criteria.

Nevertheless, the basic format of stratocladistics serves as a useful logical template. Because stratigraphy and morphology are independent data sets, the overall likelihood of any hypothesized tree is the likelihood of the tree given the character data times the likelihood of the tree given the stratigraphic data (Edwards, 1992). This is the approach that will be taken in this paper (see also Wagner, 1998a).

CALCULATING LIKELIHOOD OF A HYPOTHESIZED TREE GIVEN THE CHARACTER DATA

Congruence is an observable datum of a character matrix that is summarized by the parsimony length. The trees of interest are treated as hypotheses to be tested here. The likelihood of a hypothesized tree length (HL) yielding a parsimony length (PL) is proportional to the probability of observing the datum (PL) given the hypothesis (true tree length) (Edwards, 1992). Thus:

$$L\ [HL \mid PL] \propto P\ [PL \mid HL]$$

P [PL | HL] cannot be calculated analytically, but simulations can be used to determine the frequencies of PL's derived from trees of known lengths. The simulations illustrated in Fig. 4 were based on six-taxon trees with 25.5-step character matrices. (Again, six-taxon trees

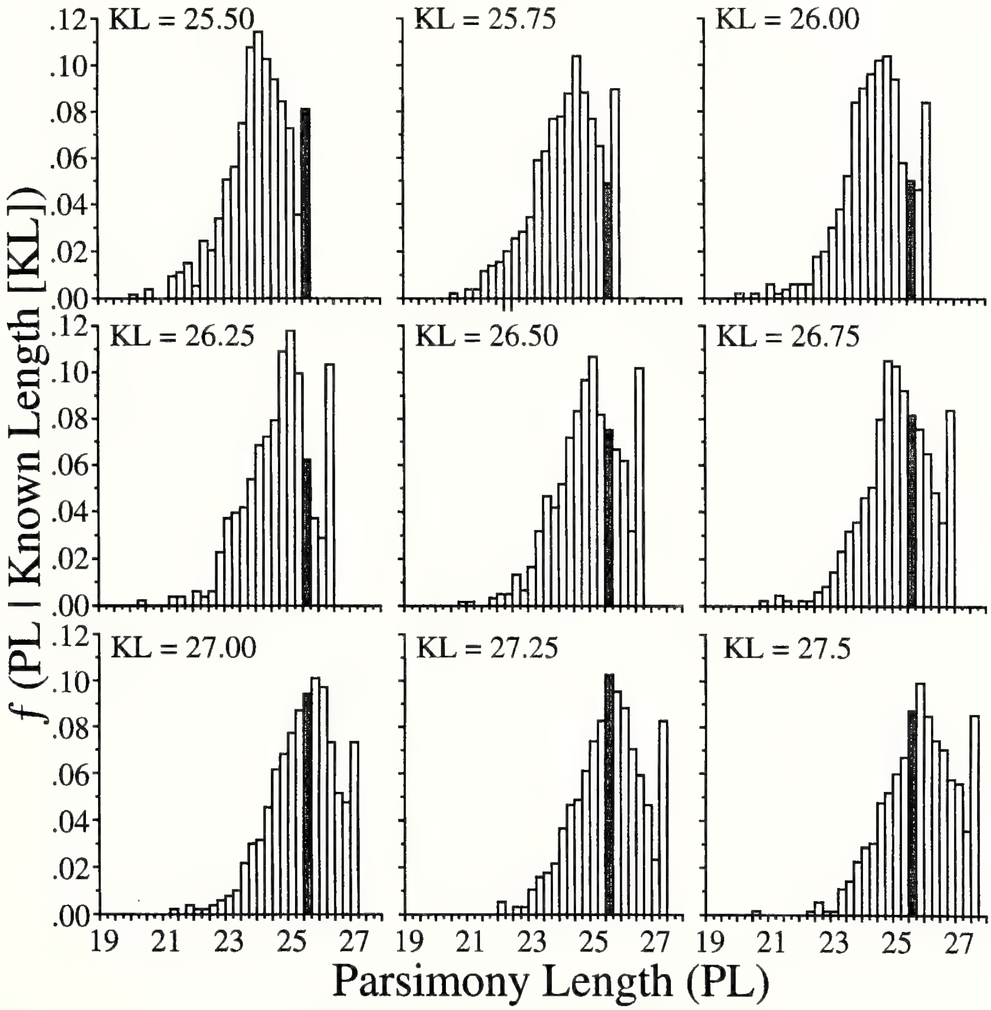


Fig. 5. The distributions of parsimony lengths given known lengths, based on simulations for six taxa with lophospiroid characters. The frequencies of matrices yielding parsimony lengths of 25.5 steps are highlighted. These match the number of steps per taxon as found for all 82 lophospiroids.

allowed use of exhaustive searches.) The frequencies of PL's given known lengths (*i. e. f* [PL | Known Length] in Fig. 5) indicate that a tree of 27 or 27.25 steps has a much higher probability of yielding a 25.5-step character matrix than does a tree of 25.5 steps. Thus:

L [HL = 27.25 | PL = 25.5] > L [HL = 25.5 | PL = 25.5]

This is best appreciated by examining the relative heights of the solid gray bars in Fig. 5, which approximate the likelihood of HL's given the observed congruence. The bar at 27.25 is the highest, indicating that this is the length most likely to have yielded the observed congruence.

One point not discussed above is that the simulations estimate P [PL | KL] only if a particular hypothesis of character evolution is assumed. Fig. 5 illustrates the results when simulated character evolution is derived from the weighting scheme (see Felsenstein, 1981). Deviations from this models resulted in longer trees lengths becoming more likely and the shortest tree length becoming less likely (Wagner, 1998a). Thus, these results represent the highest possible likelihoods for parsimony hypotheses.

We need to evaluate 82-taxon phylogenies to evaluate lophospiroid phylogenies. This is accomplished by randomly selecting 13 six-taxon clades and one four-taxon clade (derived from a separate set of simulations not illustrated here). Parsimony lengths among random six-taxon clusters within lophospiroids varied from 15.8 to 29.6 steps. Therefore, true tree lengths were randomly selected from the range of true lengths that yielded parsimony lengths of 15.8 to 29.6 steps (*i. e.* 15.8 to 54.1 steps). Note that the use of a range of true lengths means that estimated probability does not assume clock-like rates of morphologic change (*contra* Norell and Novacek, 1997).

Parametric bootstrapping (see Huelsenbeck *et al.*, 1996) then assigned parsimony lengths to each true length. Assignments used distributions such as those illustrated in Fig. 5. The true lengths and parsimony lengths of the 14 subclades are then summed, which yields the likelihood distribution for hypothesized tree lengths given 82 taxa and lophospiroid character data (Fig. 6). Log-likelihoods are illustrated because the likelihoods of shorter trees are too low to be visible on a histogram.

Parametric bootstrapping never reconstructed any trees with both true and parsimony lengths of 350.5 steps. (This is not surprising, as the probability of doing so is approximately 10^{-14}, which requires several orders of magnitudes more replications than performed.) Because the likelihoods of tree lengths under 356 steps could not be estimated from parametric bootstrapping, I assigned log-likelihoods of that length (-10.415) to all shorter lengths.

Alroy (pers. comm., 1998) suggested that the likelihood estimates for the parsimony length might be too low because heuristic searches were used to estimate the initial parsimony length of 350.5 steps. However, for a hypothesized length of 350.5 to be as likely as a tree length of 370, the actual parsimony length must be around 330 steps. It seems improbable that heuristic searches could have missed by that much. Other aspects of the test *overestimate* the likelihoods of parsimony lengths (Wagner, 1998a). The test does not allow parsimony to confound homoplasy among subclades. Also, it uses the best-case model of character evolution (see above). All other models result in a lower probability of parsimony lengths equaling hypothesized lengths. These factors, coupled with the deliberate overestimate of parsimony length likelihood described above, mean that the tests used herein are quite conservative if one wishes to treat the parsimony tree as a null hypothesis.

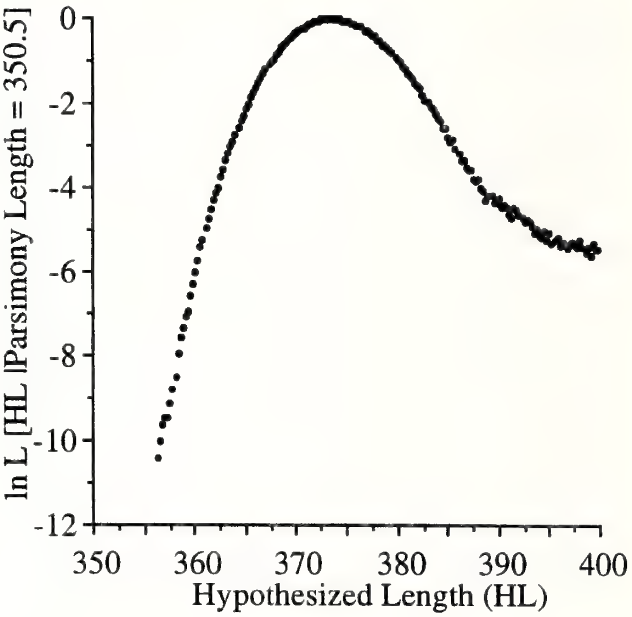


Fig. 6. Log-likelihoods of hypothesized tree lengths given a 350.5 step matrix for 82 taxa with lophospiroid characters. Note that trees 20-30 steps longer than the parsimony length are orders of magnitude more likely than are trees of 350.5 steps.

CALCULATING LIKELIHOOD OF A HYPOTHESIZED TREE GIVEN STRATIGRAPHIC DATA

Continuous Stratigraphic Data

Wagner (1995b) used continuous stratigraphic data to estimate confidence intervals for lophospiroids. Huelsenbeck and Rannala's (1997) maximum likelihood test was applied using these data (see Appendix 3). As in the 1995 analysis, the "time" scale used here is actually the number of sampled units ("horizons") per stratigraphic interval. Thus, gaps through poorly sampled intervals are more likely than are gaps through well-sampled intervals, even if both intervals are of the same duration.

Huelsenbeck and Rannala's (1997) test is modified slightly here. The most important modification is that the phylogeny is no longer considered a predictor of species' extinctions. This would be true if species evolved anagenetically. However, budding cladogenesis patterns predict only the latest possible origins of species. The parsimony tree (Fig. 1) illustrates possible examples of budding cladogenesis, as parsimony finds several plesiomorphic species known after their apomorphic sister taxa appear. ("Plesiomorphic species" are those that match inferred ancestral morphotypes.) Previous phylogenetic analyses suggest that budding cladogenesis was the predominant speciation pattern among lophospiroids (Wagner and Erwin, 1995). The exact probability of data given a hypothesized origination now is:

$$P\,[\mathrm{FKA}, \mathrm{LKA}, H \mid \mathrm{HFA}, f] = f\,(\,(\mathrm{LKA} - \mathrm{FKA})^{H-2}\, f^{H}\, e^{-f\,(\mathrm{LKA} - \mathrm{HFA})}, (H-2)!\,) \qquad \text{when } H \_ 2$$

$$= f\, e^{-f\,(\mathrm{LKA} - \mathrm{HFA})} \qquad \text{when } H = 1$$

where FKA is the first-known appearance, LKA is the last-known appearance, HFA is the hypothesized first appearance, H is the number of horizons from which the species is known, and f is the proportion of horizons within a species range from which it is sampled (amended from Huelsenbeck and Rannala, 1997: equations 2 and 4).

The likelihood of a hypothesized origination time implicit to the inferred phylogeny now is:

$$L\,[\mathrm{HFA}, \lambda \mid \mathrm{FKA}, \mathrm{LKA}, H\,] = c * \mathrm{IPR}(_{I = 1, I = \mathrm{nodes} + \mathrm{OTUs} - 1}, P\,(\mathrm{FKA}, \mathrm{LKA}, H \mid \mathrm{HFA}, \lambda)\,)$$

(amended from Huelsenbeck and Rannala, 1997: equation 5) where c is an arbitrary constant that rescales each likelihood (L), so that the maximum likelihood is 1.0 (Edwards, 1992).

Discrete Stratigraphic Data

The stratigraphic debt implicit to an inferred phylogeny implies a particular *R* (Wagner, 1998a), the likelihood of which can be evaluated (Foote, 1997). ("Sampling intensity" here denotes the average proportion of species per interval that are sampled; Foote and Raup, 1996). If a species invokes four units of stratigraphic debt, then the phylogeny implies that the species was sampled in its fifth unit after diverging from its closest sampled relative. The phylogeny therefore implies a sampling intensity of 0.2 (*i. e.* one find in five tries). For an entire phylogeny, 90 units of stratigraphic debt distributed among ten species suggests an average sampling intensity of 0.1. Because phylogenies infer latest necessary divergences (Smith, 1988), stratigraphic debt gives the minimum implied gaps. A simple analytic estimate therefore is biased toward overestimating *R*, especially on large phylogenies (Wagner, 1998a), so *R* is best estimated using simulations.

Stratigraphic debt was calculated using the substages marked in Fig. 1. For the parsimony tree, there are 102 units of stratigraphic debt. Stratigraphic debts of 102 for 82 species (two of which are present in the earliest substage) imply an $R = 0.33$. (Note that an analytic $R = f\,(80, 182) = 0.44$ is substantially greater.)

Taxon ranges reflect both *R* and durations (which reflects extinction intensity, μ) (Sepkoski, 1975; Foote and Raup, 1996). As sampling becomes worse, ranges will decrease regardless of actual durations. In the extreme case of nearly infinitesimal *R*, the few observed species will each be known from single localities and all species will have ranges of one unit. Such clearly is not the case for lophospiroids (Fig. 7A). The most likely hypothesis for observed lophospiroid ranges is $R = 0.68$ per substage (Fig. 7B, with $\mu = 0.52$ per substage). Given the *R* associated with given

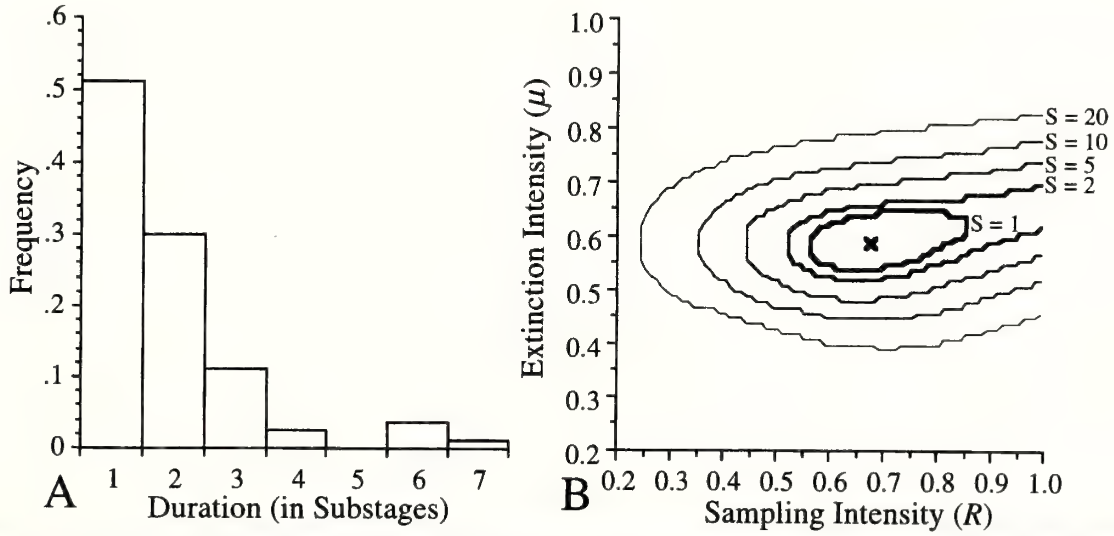


Fig. 7. Estimates of the likelihood of sampling intensities (*R*) and extinction intensities (μ). A. Distributions of observed ranges, with ranges measured by the major divisions shown in Fig. 1. B. Log-likelihoods for hypotheses that particular combinations of μ and *R* yielded the distribution shown in A. The maximum likelihood estimate is $R = 0.68$ per substage and $\mu = 0.52$ per substage.

amounts of stratigraphic debt, it now is possible to assess the likelihood of any given amount of stratigraphic debt (Fig. 8).

RESULTS

BASIC ASPECTS OF THE ALTERNATIVE TREES

Likelihoods were calculated using both methods for 100,000 trees from several different islands. Both methods found strongest support for the same tree (Fig. 9; hereafter, the ML tree). Traditional tree statistics are presented in Table 1 and the figure captions.

Table 1. Properties and statistics of alternative phylogenetic hypotheses. (HG, hypothesized gaps in horizon-level sampling; HL, hypothesized tree length; L [H | r], likelihood of a hypothesis given the measurable data; ML, maximum likelihood; Pars, parsimony; PL, parsimony reconstruction of the character matrix; SD, hypothesized stratigraphic debt, with debt measured in the same stratigraphic units as "Ranges").

Tree	HL	L [HL | PL]	SD	L [SD | Ranges]	L [HG | Horizons]
Pars	350.50	$<3 \times 10^{-5}$	102	5.95×10^{-6}	1.04×10^{-211}
ML	378.37	0.598	27	0.252	4.96×10^{-43}

The ML tree is generally similar to the parsimony tree (Fig. 1) and also to the estimates provided in Wagner (1995a). *Trochonema* is again placed among *Trochonemella* spp., whereas other taxa previously assigned to the Trochonematidae (*e. g. Eunema, Gyronema, Proturritella*) are derived separately from traditional lophospirids. This corroborates previous several previous hypotheses (*e. g.* Ulrich and Scofield, 1897; Wenz, 1938; Knight *et al.*, 1960; Erwin, 1990).

The ML tree (as the CIS tree before it) derives the problematic *Lophospira serrulata* directly from the contemporaneous *L. perangulata.* That species was previously linked by parsimony to the clade of *L. rectistriata*; here, parsimony links it to the *Trochonemella-Trochonema* clade. *L. serrulata* shares several homoplasies with other species regardless of where it is placed, including open coiling of the gerontic whorls, pronounced sutural and umbilical carinae, and sharp right and left ramp carinae. Notably, these features are shared with species known from the same sediments, such as *Trochonema umbilicata, Trochonemella notablis, L. helicteres, and Eunema strigillata.* These features are also homoplastic among these same taxa, which makes the assessment of these features as homoplastic on *L. serrulata* all the more plausible.

Multicharacter homoplasies suggest that characters did not evolve independently. However, the homoplastic characters appear in different orders among the different lineages (*i. e.* morphotype 11 might be derived from 00 → 01 in some cases, 00 → 10 in others, and directly from 00 in still other cases). Also, the characters in question all vary freely in other parts of the clade. Finally, these characters sometimes present the only discernible differences among morphospecies. The initial assumptions of character independence almost certainly are violated, albeit "fuzzily." Sophisticated likelihood tests examining varying degrees of interdependence among characters offer one solution for this problem. However, it bears stressing that the likelihood method used here recovers a pattern that violates the initial assumptions whereas parsimony does not.

Regardless of the implications for character coding,

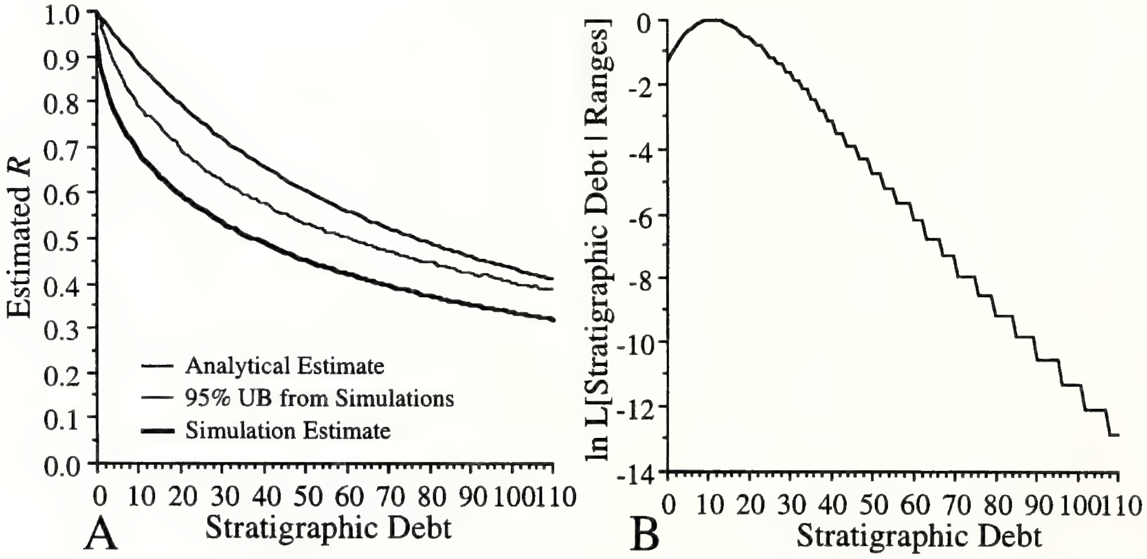


Fig. 8. A. Estimated sampling intensity (R) given stratigraphic debt distributed among 82 species (with two present in the first interval). Shown are the analytic solution, the average from 1,000 simulations and the 95%. B. Log-likelihoods of stratigraphic debts (see Fig. 7).

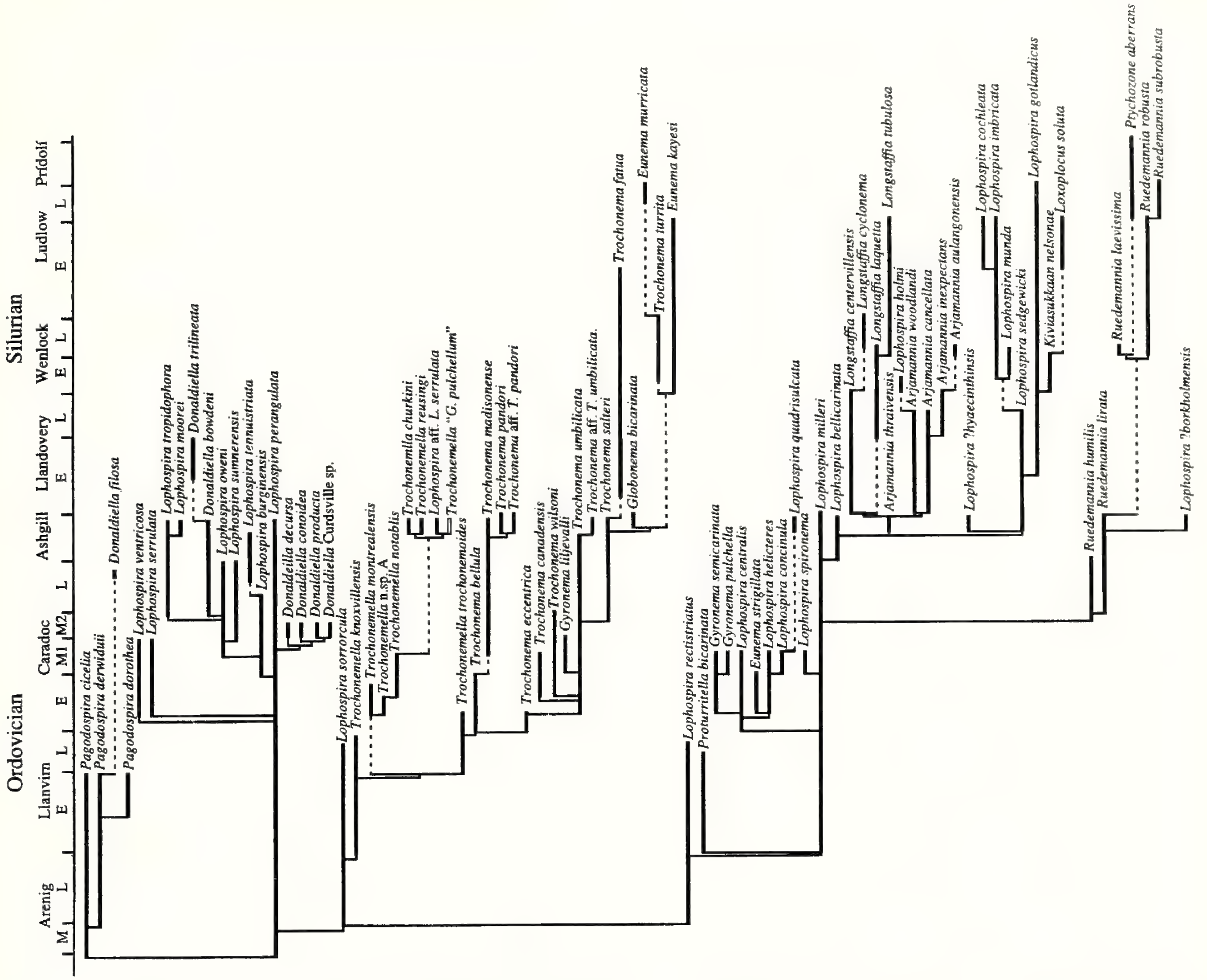

Ordovician
Silurian
Arenig
M
L
Llanvirn
E
L
Caradoc
E
M1
M2
L
Ashgill
Llandovery
E
L
Wenlock
E
L
Ludlow
E
L
Prídolí
Pagodospira cicelia
Pagodospira derwiduii
Donaldiella filosa
Pagodospira dorothea
Lophospira ventricosa
Lophospira serrulata
Lophospira tropidophora
Lophospira moorei
Donaldiella trilineata
Donaldiella bowdeni
Lophospira oweni
Lophospira sumnerensis
Lophospira tennuistriata
Lophospira burginensis
Lophospira perangulata
Donaldeilla decursa
Donaldiella conoidea
Donaldiella producta
Donaldiella Curdsville sp.
Lophospira sororcula
Trochonemella knoxvillensis
Trochonemella montrealensis
Trochonemella n.sp. A
Trochonemella notablis
Trochonemilla churkini
Trochonemella reusingi
Lophospira aff. L. serrulata
Trochonemella "G. pulchellum"
Trochonemella trochonemoides
Trochonema bellula
Trochonema madisonense
Trochonema pandori
Trochonema aff. T. pandori
Trochonema eccentrica
Trochonema canadensis
Trochonema wilsoni
Gyronema liljevalli
Trochonema umbilicata
Trochonema aff. T. umbilicata
Trochonema salteri
Globonema bicarinata
Trochonema fatua
Eunema murricata
Trochonema turrita
Eunema kayesi
Lophospira rectistriatus
Proturritella bicarinata
Gyronema semicarinata
Gyronema pulchella
Lophospira centralis
Eunema strigillata
Lophospira helicteres
Lophospira concinula
Lophospira quadrisulcata
Lophospira spironema
Lophospira milleri
Lophospira bellicarinata
Longstaffia centervillensis
Longstaffia cyclonema
Longstaffia laquetia
Longstaffia tubulosa
Arjamannia thraivensis
Lophospira holmi
Arjamannia woodlandi
Arjamannia cancellata
Arjamannia inexpectans
Arjamannia aulangonensis
Lophospira ?hyacinthinsis
Lophospira cochleata
Lophospira imbricata
Lophospira munda
Lophospira sedgewicki
Lophospira gotlandicus
Kiviasukkaan nelsonae
Loxoplocus soluta
Ruedemannia humilis
Ruedemannia lirata
Ruedemannia laevissima
Ptychozone aberrans
Ruedemannia robusta
Ruedemannia subrobusta
Lophospira ?borkholmensis

Fig. 9. Maximum likelihood estimate of lophospiroid phylogeny. The tree posits 378.4 steps (CI = 0.293; RI = 0.784) and 25 units of stratigraphic debt, with debt units based on the substages marked on the time scale. The tree is considered more likely not simply based on stratigraphic debt (Fig. 10) and continuous stratigraphic data (Table 1), but also on morphologic data (Fig. 8). As in Fig. 1, solid lines show estimated phylogenetic links and dashed lines denote ghost lineages or taxa.

the overall pattern is consistent with a hypothesis of strong functional or ecological convergence among these taxa. (Lophospiroids lacking these features are known from the same strata, so the characters cannot be dismissed simply as ecophenotypic variants.) Returning to the particular question of *Lophospira serrulata*, that species retains many plesiomorphic features. Small specimens are very similar to *L. perangulata*, save that the medial lira of the sinus keel is serrated and a prominent right ramp carina is present. (Note that "sinus keel" is used here instead of selenizone, as the latter term is used to denote a morphogenetic artifact of a slit. Sinus keels appear long before slits and the feature represents a separate homology that happens to be in the same location as a slit.) If the additional synapomorphies realized on larger specimens are associated with a functional complex, then *L. serrulata* might be separated from *L. perangulata* by fewer evolutionary innovations than implied by the character coding.

Parsimony links *Donaldiella bowdeni* and other high-spired lophospiroids to trochiform species such as *Lophospira burginensis* which differs from the trees presented by Wagner (1995b). The ML tree differs by (1) placing only *D. bowdeni* and the Silurian *D. trilineata* in that clade, and (2) considering the characters associated with the high-spired morphology to be secondarily derived rather than primitive. All trees suggest that the earliest lophospiroids were high-spired, corroborating Grabau's (1922) hypothesis about lophospiroid origins. However, the ML tree corroborates Ulrich and Scofield's (1897) hypothesis that *D. bowdeni* was derived from *L. oweni* rather than from older high-spired species.

LIKELIHOOD RATIO TESTS

Despite being nearly 10% longer than the most-parsimonious tree, the ML tree is orders of magnitude more likely than the parsimony tree given morphologic data alone (Table 1). (Again, the likelihood was calculated assuming that character evolution matched the weighting scheme of parsimony.) If variable rates of character change are used or if patterned homoplasies are included (both of which are biologically realistic), then shorter trees become even less likely whereas longer ones become more likely. The only model of evolution yields most likely lengths greater than 370 steps (*i. e.* the approximate most likely length gives a parsimony length of 350.5 steps among independently evolving lophospiroid characters): one in which there is no homoplasy. However, this hypothesis is falsified by the demonstration of incompatibility among lophospiroid characters. (In essence, this also falsifies the abductive predication of the parsimony proposition "if phylogenetic topology, then maximum congruence topology").

Likelihood ratio tests can be used to evaluate the relative support for competing hypotheses. The test statistic is:

$$\delta = 2 * (\ln L_1 - \ln L_\varnothing)$$

where $L_\varnothing$ is the likelihood of the null hypothesis (here, the parsimony tree) and L_1 is the likelihood of the test hypothesis (here, the likelihood tree) (Sokal and Rohlf, 1981: 695). The results of this test are given in Table 2. Ordinarily δ is evaluated using a chi-square distribution. Goldman (1993) cautioned against doing so when evaluating phylogenies and suggested using simulations to determine the distribution of δ instead. However, simulated δ-distributions differ from a chi-square distribution at low δ-values, but not at high ones (see Wagner, 1998a). The extremely high δ-values indicate that both morphologic and stratigraphic data provide significantly more support for the ML tree than they do for the parsimony tree.

Table 2. Log-likelihood ratio tests contrasting the parsimony estimate with the maximum likelihood estimate. "δ" gives the ratio test statistic and is evaluated using a Chi-square distribution.

	ln L[tree | PL & Strat. Data]			
Stratigraphic Likelihood	Parsimony	ML	δ	p
Stratigraphic Debt	-22.45	-1.89	41.11	1.44×10^{-10}
Horizon Data	-496.22	-97.92	796.59	2.97×10^{-175}

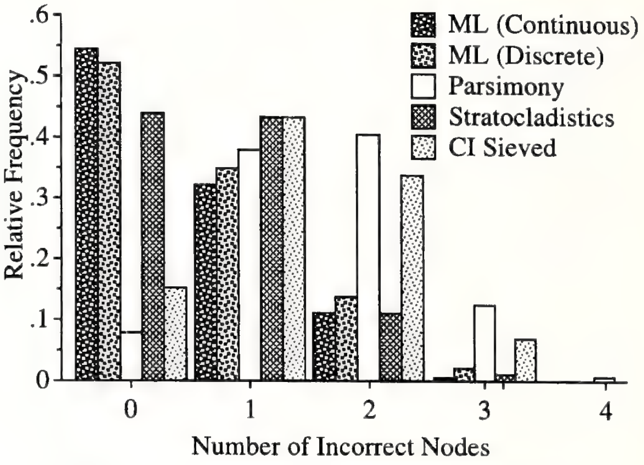


Fig. 10. Performance of maximum likelihood methods. "Continuous" uses the method of Huelsenbeck and Rannala (1997) to calculate stratigraphic likelihood; "Discrete" uses the method of Foote (1997) to calculate stratigraphic likelihood. The simulations used the "variable" model of character evolution, but the maximum likelihood estimates for morphologic change assumed an equiprobable model. Nevertheless, both methods outperformed parsimony, stratocladistics, and sieving with confidence intervals.

THE EFFICACY OF MAXIMUM LIKELIHOOD METHODS

An important consideration when contrasting different methods is relative performance under simple cases (Felsenstein, 1981, 1984). To this end, I conducted an additional set of simulations that contrasted the relative efficacy of the two maximum likelihood methods with that of parsimony, stratocladistics, and confidence interval sieving. Simulations were conducted using the variable rate model of character evolution described above (see Fig. 4). Note that the model of character change did not match the assumptions of the likelihood estimates of morphologic change. In addition, simulated sampling was made more variable, with sampling densities and sampling opportunities varying from interval to interval.

Both methods perform far better than does parsimony (Fig. 10), and both also outperform the SCI, CIS, and stratocladistics. Huelsenbeck and Rannala's (1997) method does somewhat better than does the debt-likelihood test. This is unsurprising because Huelsenbeck and Rannala's (1997) method can distinguish the relative importance of particular gaps whereas the former cannot. Interestingly, stratocladistics frequently derived the same trees for simulated clades as did maximum likelihood. The stratocladistic and CIS trees were both very similar to the maximum likelihood trees with lophospiroid data. The general congruence among these methods and the superiority of these methods over parsimony in simplified simulations both suggest that the ML estimate to be a better summary of lophospiroid evolution than is the parsimony tree.

DISCUSSION

EVOLUTIONARY IMPLICATIONS OF THE PHYLOGENETIC ESTIMATES

Accurate phylogenies are premises of many tests of macroevolutionary hypotheses (Harvey and Pagel, 1991). When presented with rival estimates of phylogeny, it is important to explore whether the topologic differences affect general macroevolutionary interpretations (Donoghue and Ackerly, 1996; Wagner, 1997).

SPECIATION PATTERNS

Initial phylogenetic estimates suggested that budding cladogenesis was the most common speciation pattern within lophospiroids (Wagner and Erwin, 1995). This study also found a positive association between species' durations and numbers of apparent descendant species. Few patterns were found consistent with either bifurcating cladogenesis or anagenesis, both of explicitly predict that ancestral and derived species did not co-occur. (Note that a "species" here is a unique combination of character states.) Budding cladogenesis does not demonstrate any particular speciation process. However, it is consistent with processes such as peripheral isolation (Mayr, 1963), shifting-balances (Wright, 1931; but see Provine, 1986), and punctuated equilibrium (Gould, 1982) and inconsistent with processes such as selective divergence (Darwin, 1859) and vicariance (Brooks and McLennan, 1991).

Both the parsimony and ML trees corroborate the original conclusions about speciation patterns. There are 18 taxa with plesiomorphic sister species on the parsimony tree. In 15 of those cases, the plesiomorphic taxon co-occurs with the derived sister taxon. There are 49 species with plesiomorphic sister species on the ML tree, 36 of which co-occur with their putative ancestor. On both trees, significantly more cases are consistent with budding cladogenesis than with anagenesis or bifurcating cladogenesis (parsimony: $p = 1.32 \times 10^{-3}$; ML: $p = 1.42 \times 10^{-4}$, based on a standard binomial test assuming a 50:50 distribution). However, one major difference exists. The parsimony tree posits no species with multiple descendants whereas the ML tree posits several species with two or more descendants. Moreover, there is a strong association between duration and the apparent number of descendants on the ML tree (Kendall's $\tau = 0.391$; $p = 2.44 \times 10^{-7}$; Sokal and Rohlf, 1981: 429). Models predicting budding cladogenic patterns are generally consistent with the pattern shown by the ML tree. However, it is not clear that any speciation models predict the pattern shown by the parsimony tree, where species such as *Lophospira perangulata* produce a single daughter taxon early and then persist many millions of years with no additional daughter taxa.

TRENDS

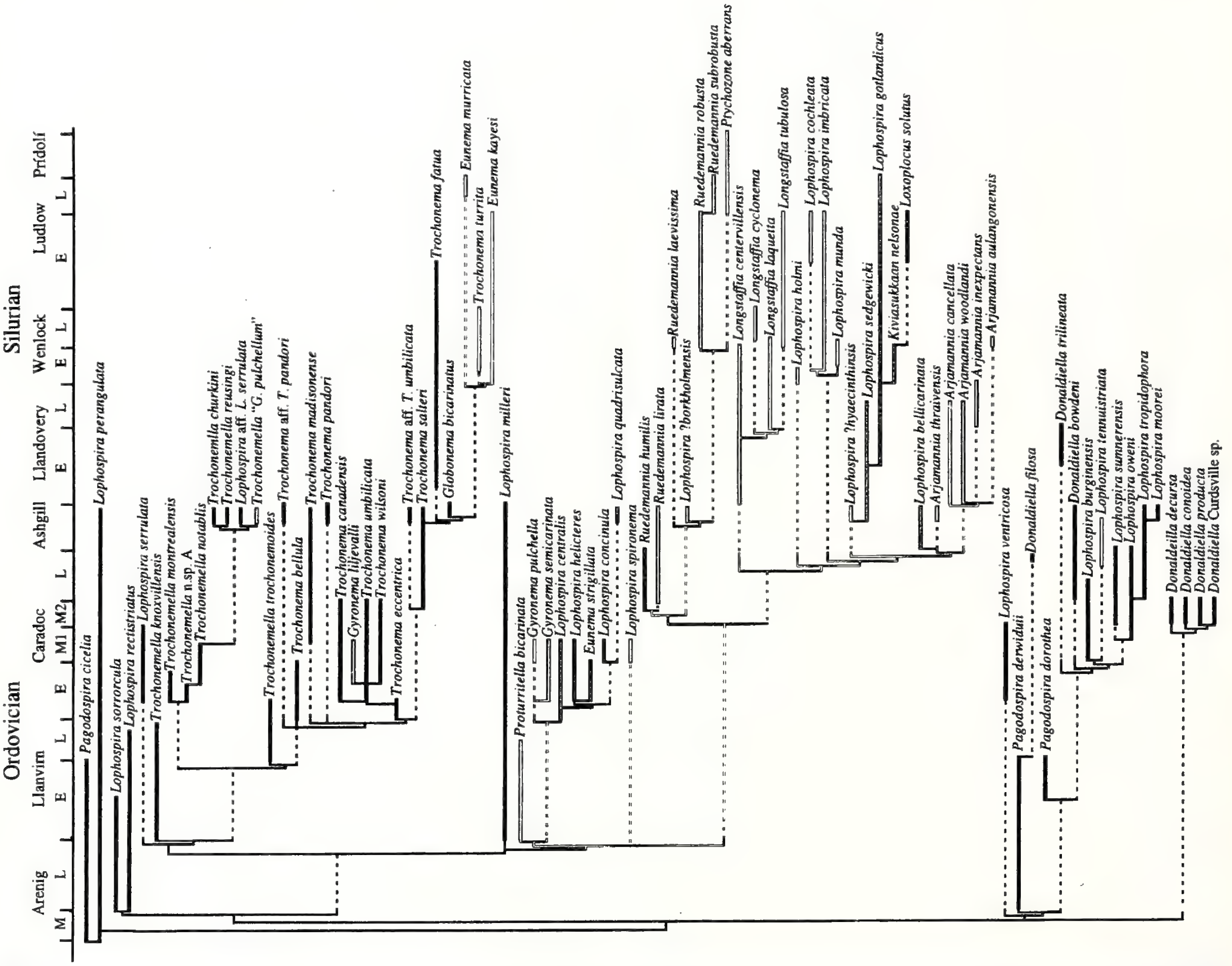
Wagner (1996) documented active trends for several shell features among Ordovician-Silurian gastropods, including lophospiroids. One of these trends, the reduction of the sinus, is clearly observable among lophospiroids. Early species such as *Lophospira perangulata* have wide, deep sinuses that curve continuously back toward the sinus keel. Similar sinuses are retained among species classified as *Donaldiella* and in some *Lophospira* subclades (*e. g.* the *L. burginensis* clade). However, reduction of the sinus happens in parallel among derived *Trochonemella* (*i. e.* the clade including *T. montrealensis* and derived *Trochonema* (*i. e.* the clade including *T. umbilicata*). Note that the common ancestor of those two clades (*i. e.* species similar to *Trochonemella knoxvillensis* and *T. trochonemoides*), retained a *L. perangulata*-like sinus. A similar pattern of sinus reduction is observed among species in the clade including *L. milleri*). It should be noted that the trend as described at this level can be inferred from the parsimony and ML trees.

Additional parallelisms concerning the sinus exist on both estimated trees. In the case of derived *Trochonema* and the clade including *Lophospira centralis*, sinuses lose most of their curvature and retreat nearly straight to the sinus keel. A parallel reduction of the sinus keel is observed within both clades. Lophospiroids primitively possess a trilineate sinus keel, with a strong, sharp, medial lira bordered by two sharp and somewhat weaker peripheral lira. Broader analyses [Wagner, in press (a)] imply that the peripheral lirae of lophospiroids are homologous with the peripheral lira of other early Murchisoniinae (*e. g. Hormotoma* and *Eotomaria*) whereas the medial lira is a synapomorphy of early lophospiroids. The peripheral lirae are lost on *Trochonema* and twice lost within the *L. centralis* clade (*i. e. Eunema* and *Gyronema* spp.). The peripheral lirae also are lost in *Proturritella* and the *Kiviasukkaan-Loxoplocus* clade. Thus, the single peripheral keel of species previously assigned to the Trochonematoidea (*e. g.* Knight *et al.*, 1960) appears to be the medial lira of primitive lophospiroids. The reduction of the sinus keel precedes the reduction of the sinus among *Trochonema* spp., because species such as *T. bellula* and *T. pandori* possess only the medial lira but retain curved and fairly wide sinuses. Conversely, the reduction of the sinus precedes the reduction of the sinus keel among the *L. centralis* clade, because species such as *L. centralis* and *L. helicteres* possess shallow, straight sinuses but retain a trilineate sinus keel. Similar mosaic patterns of sinus and sinus-keel reduction are observed within other gastropod clades [Wagner, in press (a)]. Thus, the characters clearly are independent homologues in that a change in one does not necessitate an immediate change in the others.

However, the overall pattern suggests some sort of biological (*e. g.* functional) association.

Bandel and Geldmacher (1996) have stated that species such as *Trochonema* and *Eunema* could not be derived from ancestors with sinuses and/or slits and thus could not be lophospiroids. However, early *Trochonema* spp. possess a lophospiroid sinus that is reduced or entirely lost in derived species. *Eunema* spp. retain a derived sinus observed in species previously assigned to *Lophospira* (*e. g. "L." centralis*). Sinus reduction obviously was common among Ordovician and Silurian lophospiroids. A slit is an uncommon feature among lophospiroids, but likely evolved at least three times within the clade. Derived *Trochonemella* spp. such as *T. notablis* are one such group of taxa, but primitive species such as *T. knoxvillensis* and *T. trochonemoides* clearly did not possess slits. Thus, whether *Trochonema* or *Eunema* could have slit-bearing ancestors is irrelevant, as neither ML nor parsimony trees posit slit-bearing ancestors for either taxon.

Parsimony and ML trees suggest very different patterns of ornament evolution. The increasing number of ornate gastropod taxa over the Phanerozoic is a well-documented trend (*e. g.* Vermeij, 1977). Ornament is uncommon among lophospiroids until the Late Ordovician. Conversely, most Silurian lophospiroids had at least some ornament. The parsimony tree suggests that ornament arose four times and was subsequently lost four times (Fig. 11). (The reconstructions on the parsimony tree are unambiguous and thus not affected by optimization assumptions.) Parsimony also suggests that ornate taxa were substantially more diverse in the Middle Ordovician than sampling alone would imply. Hypothesized ornate range exten-


Ordovician
Silurian
Arenig
M
L
Llanvirn
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Caradoc
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M1
M2
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Ashgill
Llandovery
E
L
Wenlock
E
L
Ludlow
E
L
Přídolí
Pagodospira cicelia
Lophospira perangulata
Lophospira sorrorcula
Lophospira rectistriatus
Lophospira serrulata
Trochonemella knoxvillensis
Trochonemella montrealensis
Trochonemella n.sp. A
Trochonemella notablis
Trochonemella churkini
Trochonemella reusingi
Lophospira aff. L. serrulata
Trochonemella "G. pulchellum"
Trochonemella trochonemoides
Trochonema aff. T. pandori
Trochonema bellula
Trochonema madisonense
Trochonema pandori
Trochonema canadensis
Gyronema liljevalli
Trochonema umbilicata
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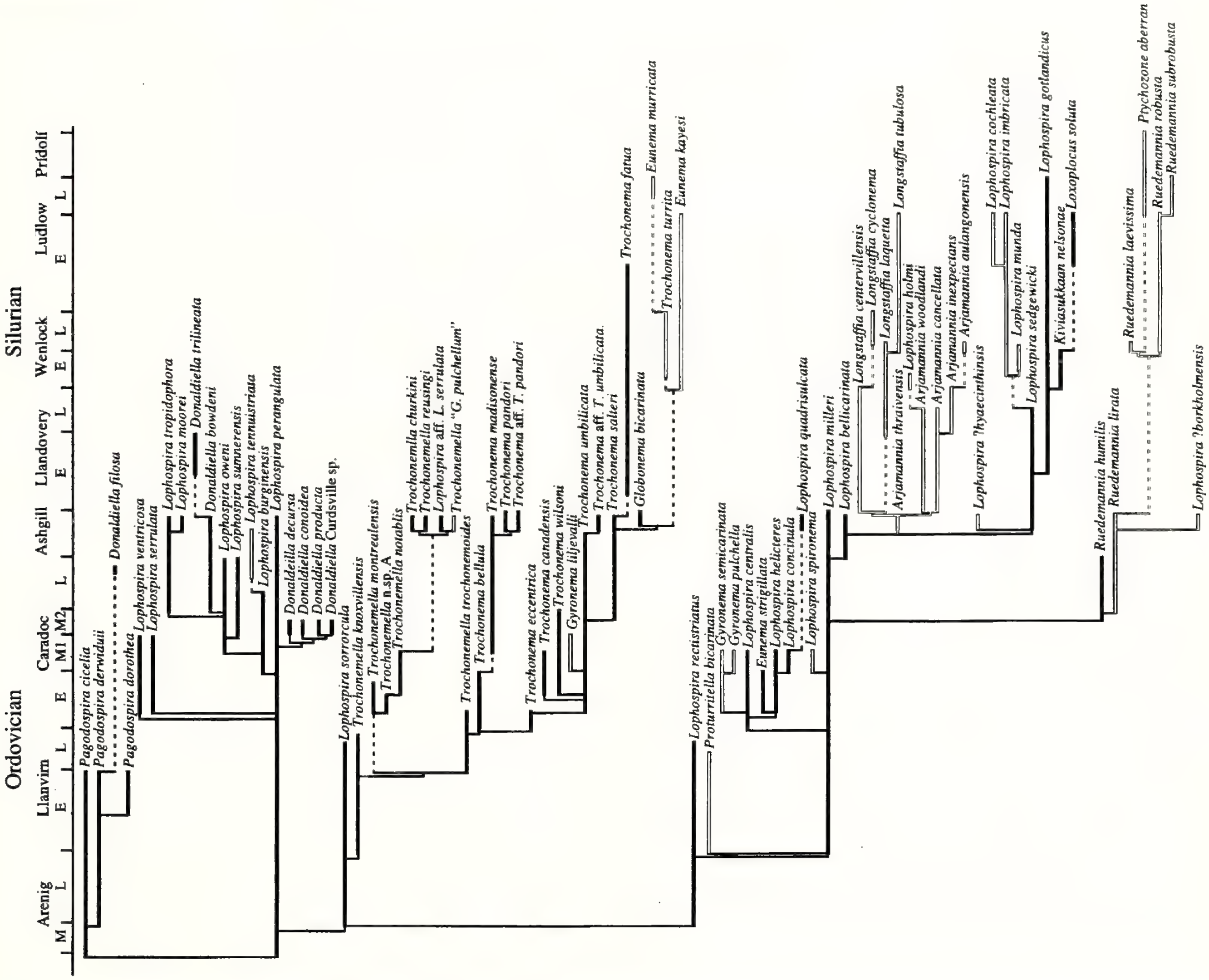
Gyronema pulchella
Gyronema semicarinata
Lophospira centralis
Lophospira helicteres
Eunema strigillata
Lophospira concinula
Lophospira quadrisulcata
Lophospira spironema
Ruedemannia humilis
Ruedemannia lirata
Ruedemannia laevissima
Lophospira ?borkholmensis
Ruedemannia robusta
Ruedemannia subrobusta
Ptychozone aberrans
Longstaffia centervillensis
Longstaffia cyclonema
Longstaffia laqueta
Longstaffia tubulosa
Lophospira holmi
Lophospira cochleata
Lophospira imbricata
Lophospira munda
Lophospira ?hyaecinthinsis
Lophospira sedgewicki
Lophospira gotlandicus
Kiviasukkaan nelsonae
Loxoplocus solutus
Lophospira bellicarinata
Arjamannia thraivensis
Arjamannia cancellata
Arjamannia woodlandi
Arjamannia inexpectans
Arjamannia aulangonensis
Lophospira ventricosa
Pagodospira derviduii
Donaldiella filosa
Pagodospira dorothea
Donaldiella trilineata
Donaldiella bowdeni
Lophospira burginensis
Lophospira tenuistriata
Lophospira sumnerensis
Lophospira oweni
Lophospira tropidophora
Lophospira moorei
Donaldeilla decursa
Donaldiella conoidea
Donaldiella producta
Curdsville sp.

Fig. 11. Patterns of ornament evolution as posited by parsimony. Black branches denote primitively inornate species, gray branches denote secondarily inornate species, and white branches denote ornate species. Note that the tree posits a much earlier divergence of ornate species than observed in the fossil record, resulting in hypotheses of many ornate ghost taxa (*i. e.* white dashed lines).

sions (Fig. 11; dashed white lines) represent approximately one-quarter of the inferred diversity during the Llanvirn. However, only one ornate species is known before the Middle Caradoc. Parsimony also implies that several inornate Ordovician species are secondarily inornate. Suspiciously, ornate relatives always appear after the "secondarily" inornate taxa.

The ML tree suggests that ornament arose nine times (Fig. 12), which implies a frequency of change of 0.098 per branch. This is over twice the typical frequency (0.040 per branch). However, ornament apparently is locally conservative, as there are no apparent reversals in subclades of up to seven ornate species. This pattern suggests a driven trend (*i. e.* the biased production of a particular morphologic type; McShea, 1994). Despite the low sample size, a G-test on exact binomial probabilities rejects any hypothesis of gains:losses ratios lower than 77:23. Vermeij (1977) hypothesized that increasing predation pressures drove a trend towards greater proportions of ornate gastropods. Taxic data suggest that this trend was well underway by the Devonian (Signor and Brett, 1984). Lophospiroids suggest that the trend began by the Late Ordovician, implying that either (1) predation pressures began earlier than previously suspected, or (2) the trend was driven (at least initially) by factors other than predation.

There are reasons why parsimony would have problems reconstructing Fig. 12. First, simulation studies indicate that frequencies of change half that of ornament characters will induce errors in parsimony (Mooers *et al.*,


Ordovician
Silurian
Arenig
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Llanvirn
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Caradoc
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M1
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Ashgill
Llandovery
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Wenlock
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Ludlow
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Pridoli
Pagodospira cicelia
Pagodospira derwiduii
Pagodospira dorothea
Donaldiella filosa
Lophospira ventricosa
Lophospira serrulata
Lophospira tropidophora
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Trochonemella notablis
Trochonemella churkini
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Trochonemella "G. pulchellum"
Trochonemella trochonemoides
Trochonema bellula
Trochonema madisonense
Trochonema pandori
Trochonema aff. T. pandori
Trochonema eccentrica
Trochonema canadensis
Trochonema wilsoni
Gyronema liljevalli
Trochonema umbilicata
Trochonema aff. T. umbilicata
Trochonema salteri
Trochonema fatua
Globonema bicarinata
Eunema murricata
Trochonema turrita
Eunema kayesi
Lophospira rectistriatus
Proturritella bicarinata
Gyronema semicarinata
Gyronema pulchella
Lophospira centralis
Eunema strigillata
Lophospira helicteres
Lophospira concinula
Lophospira spironema
Lophospira quadrisulcata
Lophospira milleri
Lophospira bellicarinata
Longstaffia centervillensis
Longstaffia cyclonema
Longstaffia laquetta
Longstaffia tubulosa
Arjamannia thraivensis
Lophospira holmi
Arjamannia woodlandi
Arjamannia cancellata
Arjamannia inexpectans
Arjamannia aulangonensis
Lophospira ?hyaecinthinsis
Lophospira cochleata
Lophospira imbricata
Lophospira munda
Lophospira sedgewicki
Lophospira gotlandicus
Kiviasukkaan nelsonae
Loxoplocus soluta
Ruedemannia humilis
Ruedemannia lirata
Ruedemannia laevissima
Ptychozone aberran
Ruedemannia robusta
Ruedemannia subrobusta
Lophospira ?borkholmensis

Fig. 12. Patterns of ornament evolution as posited by likelihood. Black branches denote primitively inornate species and white branches denote ornate species. No secondarily inornate species are posited. The evolution of ornate species per substage here matches patterns observed in the fossil record and suggests a driven trend (*sensu* McShea, 1994).

1995). Simulations further suggest that parsimony is even less successful when transition patterns are biased (Kuhner and Felsenstein, 1994; Lamboy, 1994). Second, it is impossible to code ornament characters in a way that is neutral with regard to all possible patterns of evolution. Some species possess ornament throughout the shell, but species such as *Ruedemannia lirata* and *Arjamannia thraivensis* possess ornament only on the left ramp. In some non-lophospiroids, ornament occurs only on the right ramp. Thus, the presence and absence of ornament must be scored separately for both ramps. The result is that completely ornate species share two synapomorphies instead of one; if evolution goes from completely inornate to completely ornate (*e. g. Lophospira milleri* to *Proturritella bicarinata*), then parsimony counts two steps instead of one. A third and related reason is that similar characters were coded as synapomorphic (*sensu* Sober, 1988), if only at basic levels. For example, a species with coarse, broadly-spaced ornament on the left ramp shared the presence of left ramp ornament (character 87) with a species with fine, densely-packed ornament. Additional characters (*e. g.* characters 88 and 89) then were used to distinguish the two. This assumes that it is easier to modify a character than it is to evolve it, which many workers regard as the lesser assumption (*e. g.* Hennig, 1966).

The trend toward increasing numbers of ornate species highlights a potential strength of the maximum likelihood method over parsimony. Parsimony is ill-equipped to deal with biased transitions and character transitions that cannot be neatly coded. As such, the pattern of evolution shown in Fig. 12 predicts that parsimony results such as shown in Fig. 11 should be probable. However, the maximum likelihood analysis (with the help of stratigraphic data) was able to recover a pattern of evolution that was at odds with the initial assumptions.

The effect of initial assumptions about character change has additional implications. The ML tree suggests a trend towards increasing numbers of ornate species induced by a tendency for inornate ancestors to yield ornate descendants. The parsimony tree suggests a trend driven by a very speciose ornate clade. Several workers (*e. g.* Wright, 1967; Eldredge and Gould, 1972; Stanley, 1975; Vrba and Eldredge, 1984) hold that differential speciation might induce trends and that morphologic evolution might be random with respect to those trends (*i. e.* "Wright's Rule"; Gould, 1977). By minimizing the number of times characters arose and by maximizing the number of taxa linked by those characters, parsimony might be biased towards results consistent with "species selection" rather than with driven trends. Lophospiroids offer a possible example of this bias.

RECOMMENDED TAXONOMIC REVISIONS

Phylogenetic frameworks serve as a basis for taxonomic classification, which in turn can convey historical patterns of biodiversity to non-specialists. Conveying diversity patterns taxonomically is not simple. Some workers (*e. g.* Eldredge and Novacek, 1985; Smith and Patterson, 1988) argue that only monophyletic supraspecific taxa can reflect historical diversity patterns. If so, then phylogenetic systematics (Hennig, 1966) is appropriate. However, other workers use higher taxa as proxies for species in diversity studies because higher taxa are less prone to sampling biases than are species (*e. g.* Raup, 1979; Sepkoski, 1979, 1993; Raup and Boyajian, 1988). Paraphyletic and monophyletic taxa both are excellent proxies for extant species diversities (Williams and Gaston, 1994; Andersen, 1995; Balmford *et al.*, 1996). Both simulation (Sepkoski and Kendrick, 1993; Maley *et al.*, 1997) and empirical studies (Wagner, 1995c) indicate that taxonomic schemes including paraphyletic taxa portray species-level diversity patterns as well as or better than do those using monophyletic taxa alone. Including paraphyla becomes especially important when rates of cladogenesis and/or extinction or sampling intensities vary over time (Wagner, 1995c). Thus, a taxonomic scheme designed to reflect historical diversity patterns should include paraphyletic taxa lest it be biased against depicting particular patterns.

Evolutionary systematics (Simpson, 1961) allows for paraphyletic taxa, but evolutionary systematics has no explicit, repeatable criteria for delimiting such taxa (Hull, 1967; note that the same actually is true of Hennigian systematics; Tassy, 1988). Phylogenies are nothing more than artifacts of speciation and extinction rates (Sepkoski, 1987). Such an evolutionary parameter can be used to delimit "real" groups in a statistical sense. For example, quantitative analyses of net rates of cladogenesis in primates (Purvis *et al.*, 1995) and rates of morphologic change in rostroconch molluscs (Wagner, 1997) separate paraphyletic groups from monophyletic ones. Unfortunately, demonstrating that sets of species belong to statistically distinguishable groups requires large sample sizes, much larger than is available for defining genera or probably even families. Estabrook (1986) proposed integrating phenetic dissimilarity with phylogenetic topology to delimit higher taxa. This has the advantage of being fully repeatable once criteria are made explicit. However, if rates of morphologic evolution vary over time or across a clade, then more higher taxa will unite very different numbers of species and thus will not portray historical biodiversity accurately.

Although based on the ML tree, the generic definitions presented here would be nearly identical if derived from the parsimony tree. The lack of explicit criteria for delimiting genera does not interfere with a generic revision that better reflects historical diversity patterns than current generic schemes do (Fig. 13). For example, *Lophospira*

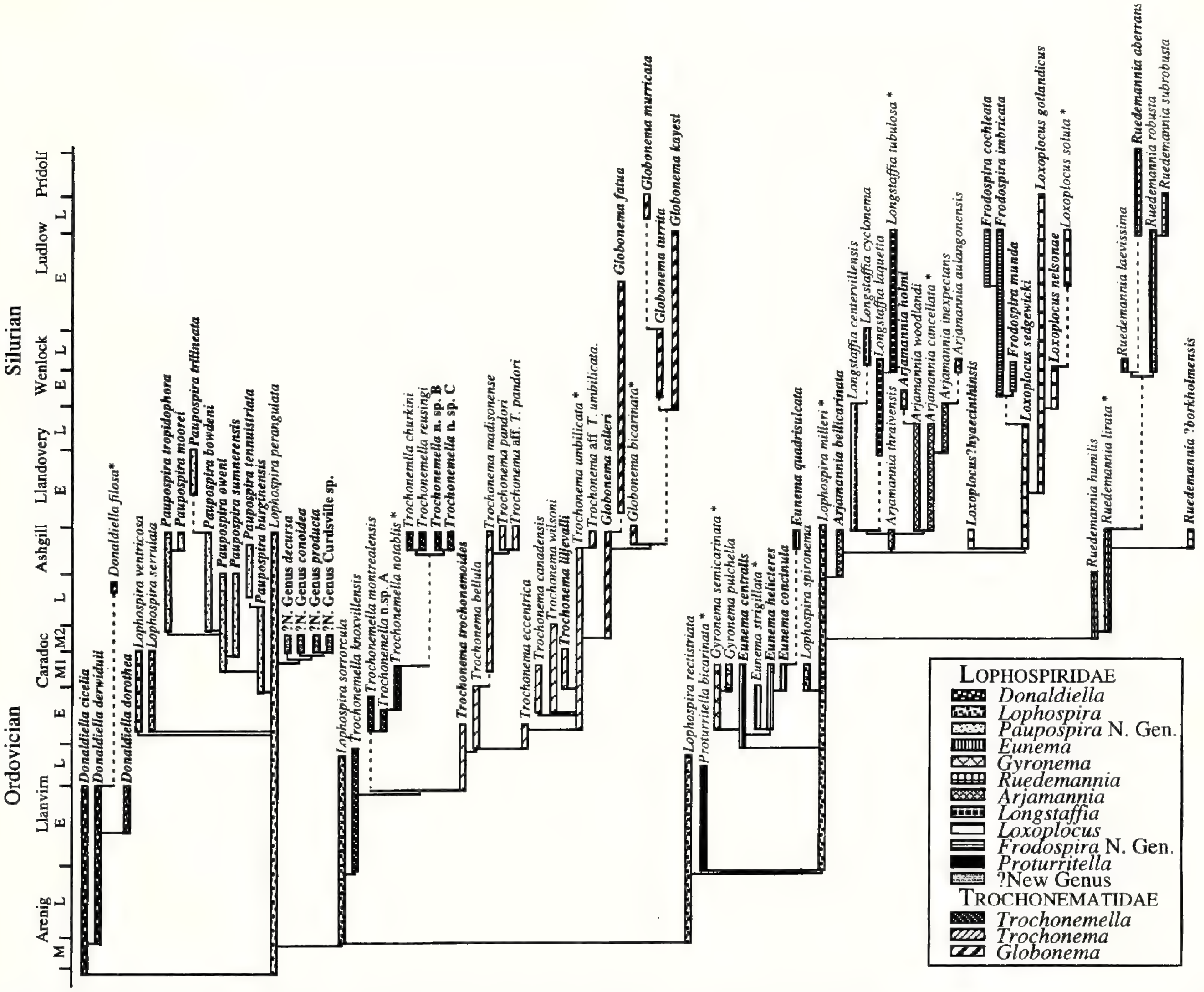
Ordovician
Silurian
Arenig M L
Llanvirn E L
Caradoc E M1 M2
Ashgill L
Llandovery E L
Wenlock E L
Ludlow E L
Pridolí
Donaldiella cicelia
Donaldiella derwiduii
Donaldiella dorothea
Donaldiella filosa*
Lophospira ventricosa
Lophospira serrulata
Pauposprira tropidophora
Pauposprira moorei
Pauposprira trilineata
Pauposprira bowdeni
Pauposprira oweni
Pauposprira sumnerensis
Pauposprira tennuistriata
Pauposprira burginensis
Lophospira perangulata
?N. Genus decursa
?N. Genus conoidea
?N. Genus producta
?N. Genus Curdsville sp.
Lophospira sorrorcula
Trochonemella knoxvillensis
Trochonemella montrealensis
Trochonemella n.sp. A
Trochonemella notablis*
Trochonemella churkini
Trochonemella reusingi
Trochonemella n. sp. B
Trochonemella n. sp. C
Trochonema trochonemoides
Trochonema bellula
Trochonema madisonense
Trochonema pandori
Trochonema aff. T. pandori
Trochonema eccentrica
Trochonema canadensis
Trochonema wilsoni
Trochonema liljevalli
Trochonema umbilicata*
Trochonema aff T. umbilicata.
Globonema salteri
Globonema bicarinata*
Globonema fatua
Globonema muricata
Globonema turrita
Globonema kayesi
Lophospira rectistriata
Proturritella bicarinata*
Gyronema semicarinata*
Gyronema pulchella
Eunema centralis
Eunema strigillata*
Eunema helicteres
Eunema concinnula
Eunema quadrisulcata
Lophospira spironema
Lophospira milleri*
Arjamannia bellicarinata
Longstaffia centervillensis
Longstaffia cyclonema
Longstaffia laqueta
Longstaffia tubulosa*
Arjamannia thraivensis
Arjamannia holmi
Arjamannia woodlandi
Arjamannia cancellata*
Arjamannia inexpecians
Arjamannia aulangonensis
Loxoplocus?hyacinthinsis
Frodospira cochleata
Frodospira imbricata
Frodospira munda
Loxoplocus sedgewicki
Loxoplocus gotlandicus
Loxoplocus nelsonae
Loxoplocus soluta*
Ruedemannia humilis
Ruedemannia lirata*
Ruedemannia laevissima
Ruedemannia aberrans
Ruedemannia robusta
Ruedemannia subrobusta
Ruedemannia ?borkholmensis
LOPHOSPIRIDAE
Donaldiella
Lophospira
Pauposprira N. Gen.
Eunema
Gyronema
Ruedemannia
Arjamannia
Longstaffia
Loxoplocus
Frodospira N. Gen.
Proturritella
?New Genus
TROCHONEMATIDAE
Trochonemella
Trochonema
Globonema

Fig. 13. Proposed taxonomic revisions. "*" denotes the type species of each genus. "*Proturritella*" is retained because additional species not available for this analysis might belong there. Formal designation of "?New Genus" is withheld pending additional analyses, because it is possible that those species are instead affiliated with *Donaldiella filosa,* the type species of *Donaldiella.* If so, then the species here placed in *Donaldiella* should be returned to Pagodospira. See Appendix 4 for necessary rediagnoses and redefinitions.

bellicarinata could be assigned to ether *Arjamannia* or *Loxoplocus*; in either case, one genus appears in the Late Caradoc whereas the other appears in the Ashgill. However, retaining all *Arjamannia* and *Loxoplocus* spp. in *Lophospira* results in a taxonomy that ignores the derivation of a diverse clade whose sister taxa (*i. e.* other derivatives of *L. milleri* such as *Ruedemannia* and the *Eunema-Gyronema* clade) are separated into one or more genera.

In some cases, the revisions simply update lophospiroid taxonomy. Many of the species analyzed here were originally classified before *Lophospira* was partitioned into additional genera. Initial diagnoses of new genera were not accompanied by exhaustive revisions of the entire clade, resulting in a highly polyphyletic *Lophospira.* This is remedied to make *Lophospira* a paraphyletic group. Other cases involve redefining and rediagnosing obviously polyphyletic genera. For example, *Eunema* previously has been diagnosed by high translation (*sensu* Raup, 1966), the absence of a sinus and a medial keel. A sinus actually is present in the type species, albeit a shallow V-shaped one that is shared with some species formerly placed in *Lophospira* (all now reclassified as *Eunema*). In the redefinition presented here, only the type species lacks a trilineate sinus keel. However, the loss of a peripheral lira represents a derived condition that happened multiple times and therefore cannot unequivocally diagnose genera.

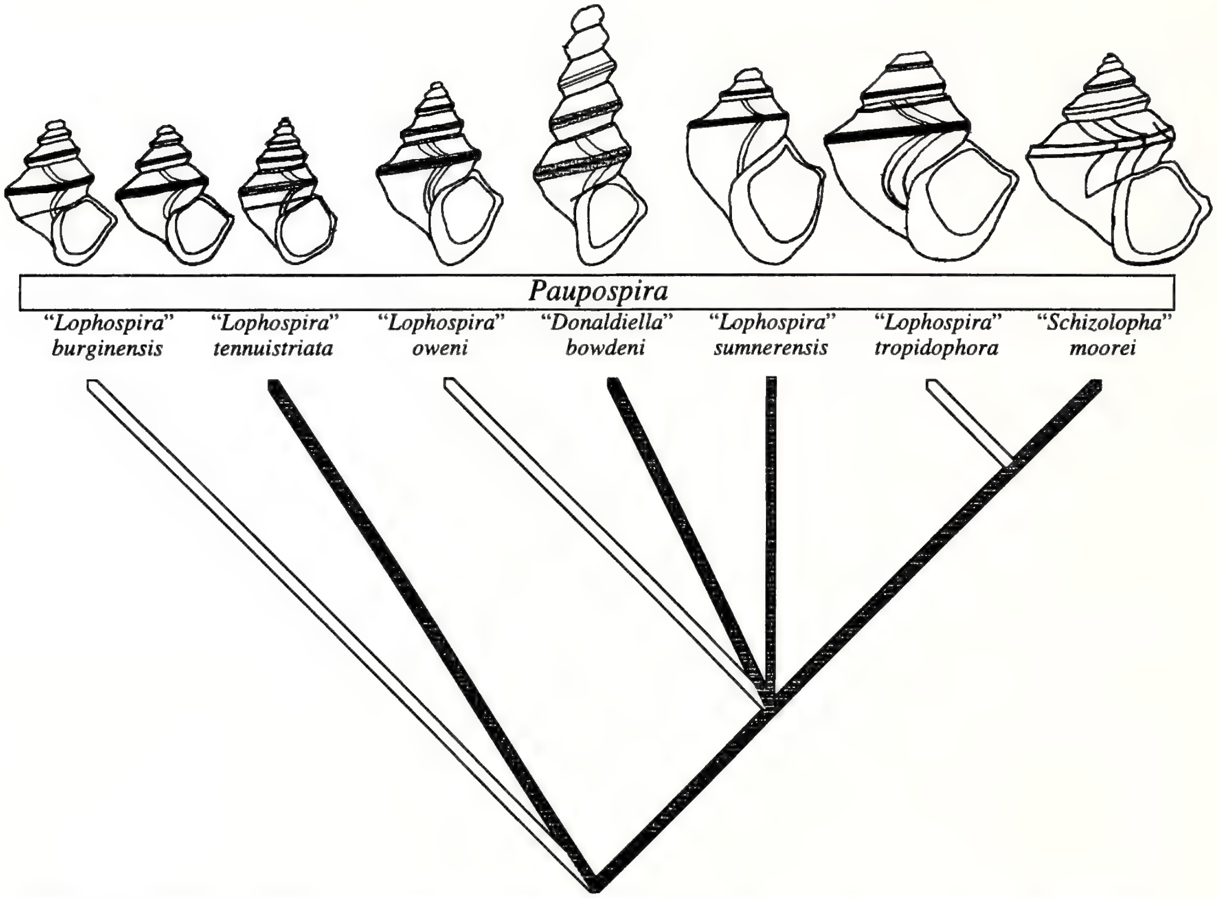

Fig. 14. Cladogram of *Paupospira* highlighting the important morphologic features of the clade. White branches denote zero-length branches (*i. e.* species identical to hypothesized ancestral morphotypes).

Patterson and Smith (1987) suggested that some diversity patterns inferred from taxic data might be artifacts of paraphyly. However, if a paraphyletic taxon's disappearance is not due to its sole species anagenetically transforming into a species placed in a different higher taxon, then its disappearance necessarily reflects the extinction of at least one species. Because paraphyletic taxa necessarily are older than their monophyletic "descendants," paraphyla often have higher standing diversities than their monophyletic "daughters" (Uhen, 1996) and thus require greater numbers of species extinctions to be terminated. For example, *Trochonemella* is paraphyletic relative to *Trochonema*, which in turn is paraphyletic relative to *Globonema*. However, the extinctions of *Trochonemella* and *Trochonema* in the Ashgill represents the loss of eight species (as well as the disappearance of particular morphotypes). *Globonema* represents two survivors, so the 2:1 casualties:survivors ratio implied by genera underestimates the 4:1 ratio implied by species. Alternative classifications fare worse. Any monophyletic definition including all members of the clade fails to register the extinction. Conversely, reduced monophyletic definitions recognizing only the clades terminated in the Ashgill fail to recognize that the group diverged during the Middle Ordovician. A new monophyletic genus, *Paupospira*, also illustrates how strictly monophyletic higher taxa can obfuscate historical patterns. That genus is nearly eliminated by the end-Ordovician extinction, but a sole survivor is known from the Llandovery. The genus' demise somewhat misleadingly now represents a "background" extinction (Jablonski, 1986).

CONCLUSIONS

Although malacologists have questioned the utility of shell characters in phylogenetic analyses, shell character data for Ordovician-Silurian lophospiroids show compatibility similar to that expected if those data do retain hierarchical (phylogenetic) signal. Nevertheless, truly hierarchical signal among the clade as a whole and geologically older taxa in particular decreases with the addition of geologically younger taxa. Phylogenetic methods incorporating stratigraphic data outperform parsimony for simulated clades with similar fossil records and the same number of characters and character states as lophospiroids. Correspondingly, the parsimony estimate of lophospiroid phylogeny posits many more gaps than plausible given the quality of the lophospiroid record.

Likelihood tests support a tree that is 30 steps longer but nevertheless is more likely based on both morphologic and stratigraphic data. Both trees suggest that budding cladogenesis was the dominant speciation pattern, and both portray trends toward increasing numbers of ornate species. However, the likelihood tree depicts a strong association between duration and proclivity at the species level whereas the parsimony tree does not. Also, the likelihood tree suggests that the ornament trend was driven by the acquisition of ornament being far more common that its loss. Parsimony suggests that ornate clades were simply more diverse that were inornate clades. At least in this example, parsimony appears biased toward results suggesting patterns consistent with "species-selection." Lophospiroids highlight the utility of gastropods as a model group for testing macroevolutionary hypotheses. However, lophospiroids also highlight the importance of developing phylogenetic methods in whose results we can be more confident.

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APPENDIX 1

CHARACTERS AND CHARACTER STATES

Character state codings follow Wagner [in press (a)]. Lophospirioids do not possess all of the states available in some multistate characters developed for that analysis; therefore, in the following list, the plesiomorphic state for lophophorids is not coded "1" in all cases, and some character states appear to be missing. The character coding has not been adjusted herein, so that lophospiroids can be easily compared with other gastropods.

1. Sinus (broad cleft in shell, culminating at presumed location of anus): (1) absent; (2) present. Weight 1.
2. Sinus depth [as described by general angle of retreat by sinus; continuous character, ranging from almost 0° to 40°; really a shape metric, as true depth is a product of angle of retreat, sinus width (character 3), and curvature of retreat (character 4)]: (1) >0°; (2) ~10°; (3) ~20°; (4) ~30°; (5) ~40°. Ordered; weight 0.25.
3. Sinus width [as described by angle passing from center of aperture through top and bottom of sinus; continuous character, with states broken into just above/below sinus apex (1), halfway between top/bottom of ramp and sinus apex (2), coinciding with top/bottom of ramp (3), and beyond top/bottom of ramp (4); coding the position of the onset of the sinus relative to the top/bottom of the ramps rather than the absolute width accommodates the fact that changes in ramp lengths and orientations also will change sinus width as an artifact; this coding scheme focuses only on differences clearly attributable to the sinus itself]: (1) just above SK; (2) between SK and RR; (3) at RR. Ordered; weight 0.5.
4. Sinus shape [describing general trend of sinus; nearly straight up to sinus apex (*i. e.* V-shaped) or curving back continuously; this describes the shape of the curve whereas sinus depth (character 2) describes the general angle of retreat]: (2) straight; (3) continuous. Weight 0.5.
5. Crenulated aperture [in which apertural margin generates "zig-zag" growth lines]: (1) absent; (2) present. Weight 1.
6. Sigma-shaped aperture: (1) absent; (2) intersection between base and alveozone curved (yielding reverse sigma) instead of planar or angular. Weight 1.
7. Prominence of growth lines (GL) [evaluated only by comparison with both different specimens known from same preservational conditions and similar specimens from different preservational conditions]: (2) weak; (3) fine sharp; (4) strong. Ordered; weight 0.5.
8. Imbricated GLs [alternating growth lines appreciably stronger than others; considered present only if imbrications obviously are patterned, as random imbrications can occur due to shell repair or on gerontic whorls of some species; growth line prominence is coded based on weaker growth lines]: (1) absent; (2) weak; (3) moderate; (4) strong. Unordered; weight 1.
9. Sinus keel (SK) width [originally thought to be morphologic artifact of a slit or a "notch," however, appearing on slitless specimens and the seemingly mythical "notch" has never been documented; continuous character, where 360(equals circumference of aperture]: (1) ~5°; (2) ~10°; (3) ~15°; (4) ~20°; (5) ~25°; (6) ~30°. Ordered; weight 0.2.
10. Peripheral lirae (PL) [two lirae on bilineate peripheral bands (or outer two on trilineate bands of lophospiroids); in some cases, might be produced by placing a slit within a medial lira (see below); however, not occuring on all species with slits and many species with peripheral lira clearly do not have slits]: (1) absent; (2) present. Weight 1.
11. PL type: (1) threads with round profile (*e. g. Ruedemannia*); (2) threads with sharp profile (*e. g. Ectomaria* or *Lophospira*). Weight 1.
12. PL strength: (2) weak (clearly visible but casting little relief); (3) moderate (casting noticeable relief); (4) strong (clearly projecting from shell). Ordered; weight 0.5.
13. Medial lira (ML) [= "notch keel" or "sinus keel"; present with peripheral lirae (character 10) only if there is ontogenetic change (character 25) or if peripheral band is trilineate; carina at the apex of the sinus]: (1) absent; (2) present. Weight 1.
14. ML type [as seen in profile]: (1) round, of equal "height" and width (*e. g. Ruedemannia*); (2) sharp, of equal "height" and width (e. g. Lophospira). Weight 1.
15. ML strength: (1) extremely weak (barely visible, casting almost no relief); (2) weak (clearly visible but casting little relief); (3) moderate (casting noticeable relief); (4) strong (clearly projecting from shell). Ordered; weight 0.33.
16. Imbricated ML: (1) consistent; (2) flaring periodically (*e. g. Lophospira serrulata*).
17. SK prominence [not equal to strength, as prominence refers to whole structure projecting from rest of shell; sometimes coincides with channel underneath peripheral band; however, species with channels and non-prominent peripheral bands and species with prominent peripheral bands and no obvious channel are both known]: (1) peripheral band not altering profile of whorl; (2) whole peripheral band projecting slightly, creating slight ridges between shell and peripheral band; (3) whole peripheral band projecting strongly, creating strong ridges between shell and peripheral band. Ordered; weight 0.5.
18. Slit: (1) absent; (2) present. Weight 1.
19. Slit depth: (1) shallow (extending <10° behind aperture, measured looking down coiling axis); (2) deep (extending ~20° behind aperture). Weight 1.
20. Maintenance of slit: (1) periodically erased or reduced, with shell material deposited within slit less frequently than on rest of shell, resulting in slit depth (and sometimes presence/absence) varying over time (*e. g. Trochonemella*; recognized in part by greater distance between lunulae than observed between growth lines); (2) continuously maintained (*e. g. "Schizolopha" moorei*). Weight 1.
21. Lunulae shape [lunulae here are growth lines within sinus keel]: (1) concentric (shallow U-shape); (4) V-shaped (*e. g. Lophospira centralis* and relatives). Weight 1.
22. Lunulae strength: (1) weaker than GLs; (2) same as GLs; (3) stronger than GLs (*i. e.* imbricated; *e. g. Trochonemella* spp.). Unordered; weight 1.
23. Imbricated lunulae type: (1) deep and obtuse; (2) serrated. Weight 1.
24. Ontogenetic change in imbricated lunulae: (1) juvenile whorls only; (2) throughout. Weight 1.
25. Reduction to monolineate SK over ontogeny: (1) absent; (2) monolineate on final whorls. Weight 1.
26. Peripheral band attitude: (1) projecting straight from shell, as seen on most species; (2) curving adapically, *e. g. Arjamannia* and relatives. Weight 1.
27. Midwhorl (MW) channel [groove underneath medial lira; often but not always associated with prominent band, and so coded separately]: (1) absent; (2) present. Weight 1.
28. MW channel strength: (1) weak; (2) strong. Weight 1.
29. SK position [described relative to aperture centroid, with 90(indicating that a plane is passing through centroid and that SK is perpendicular to coiling axis; 0(indicating that plane is parallel to coiling axis]: (2) ~100°; (3) ~90°; (4) ~80°; (5) ~70°; (6) ~60°; (7) ~50°. Ordered; weight 0.2.
30. Ramp shape symmetry [on primitive bilaterally symmetrical gastropods, right and left ramp shapes are symmetrical; derived species are asymmetrical]: (1) right rounder; (2) symmetrical; (3) left rounder. Unordered; weight 1.

31. Right ramp (RR) shape: (1) globular; (2) convex; (3) flat; (4) slightly concave; (5) concave. Ordered; weight 0.125 (reflecting both continuous nature and de-weighting for asymmetry).
32. RR and LR lengths [on primitive bilaterally symmetrical gastropods, right and left ramp lengths are equal ; lophospirds with longer right and longer left ramps are known]: (1) longer RR; (2) equal lengths; (3) longer LR. Unordered; weight 1.
33. RR length [describing angle from "top" of ramp to sinus apex, based on triangle passing through aperture centroid]: (3) ~50°; (4) ~60°; (5) ~70°; (6) ~80°; (7) ~90°. Ordered; weight 0.25.
34. LR length [see character 33]: (3) ~50°; (4) ~60°; (5) ~70°; (6) ~80°; (7) ~90°. Ordered; weight 0.25.
35. RR:LR projection [ramp projection describing angle of "rise" of ramp from sinus keel, based on plane passing through SK and aperture centroid]: (1) RR projection higher; (2) LR and RR projections equal. Weight 1.
36. RR projection [see character 35]: (5) ~60°; (6) ~70°; (7) ~80°(. Ordered; weight 0.25.
37. LR projection [see character 35]: (3) ~40°; (4) ~50°; (5) ~60°; (6) ~70°; (7) ~80°. Ordered; weight 0.125.
38. Sutural carina (SC): (1) absent; (2) present. Weight 1.
39. SC strength: (1) weak (creating only a weak profile); (2) moderate (partly filling up the suture).
40. RR carina (RRC) [strong carina usually located at top of sinus]: (1) absent; (2) present. Weight 1.
41. RRC strength: (1) weak (creating no profile); (2) moderate (roughly equal to weak-to-moderate peripheral band); (3) strong (roughly equal to strong peripheral band). Ordered; weight 0.5.
42. RRC type: (2) thin local thickening; (3) round profile; (4) sharp profile. Unordered; weight 1.
43. Ontogenetic change in RRC strength: (1) absent; (2) becoming weaker on adult whorls. Weight 1.
44. Channel beneath RC: (1) absent; (2) present. Weight 1.
45. RRC location: (1) ~75° toward suture from SK; (2) ~45° toward suture from SK. Weight 1.
46. RRC attitude: (1) carina projecting perpendicularly to RR; (2) carina curving abapically. Weight 1.
47. RRC: (1) plain thread; (2) serrated. Weight 1.
48. Shape of shell at top of right ramp [usually at suture]: (1) oblique; (2) acute, with channel underneath suture. Weight 1.
49. LR shape [see character 31]: (1) globular; (2) very convex; (3) slightly convex; (4) flat. Ordered; weight 0.167.
50. Swelling at intersection of left ramp and base [might be primitively homologous with left ramp carina (character 51), and present on earliest gastropods]: (1) absent; (2) present. Weight 1.
51. LR carina (LRC): (1) absent; (2) present. Weight 1.
52. LRC type: (1) thick contusion, but creating more distinct profile than simple swelling; (2) sharp profile. Weight 1.
53. LRC strength [see character 41]: (1) weak; (2) moderate (~ sinus); (3) strong. Ordered; weight 1.
54. LRC [see character 47; species with serrated right carinae but plain left carina exist, indicating that the two evolve independently]: (1) plain thread; (2) serrated. Weight 1.
55. Second LRC: (1) one carina; (2) two carinae (*e. g. Lophospira quadrisulcata*). Weight 1.
56. Channel beneath LRC [see character 44]: (1) absent; (2) present. Weight 1.
57. Columella thickness: (1) no thicker than rest of shell; (2) slightly thicker than rest of shell; (3) much thicker (partly filling umbilicus); (4) extremely thick (filling umbilicus). Ordered; weight 0.33.
58. Umbilical carina (UC) [carina at base of shell, circling umbilicus]: (1) absent; (2) present. Weight 1.
59. UC type: (1) thick, dull protrusion; (2) sharp extension accomodating a channel; (3) lirum with sharp profile. Unordered; weight 1.
60. UC strength [see characters 41 and 53]: (1) very weak; (2) weak; (3) strong. Ordered; weight 0.5.
61. Ontogenetic change in UC strength: (1) constant; (2) becoming weaker on adult whorls. Weight 1.
62. UC location [with larger angle indicating UC closer to coiling axis]: (1) ~120° below SK; (2) ~90° below SK. Weight 1.
63. Angle at base of columella [narrower angle indicates sharper, more siphonate base of shell]: (3) ~60°; (4) ~75°; (5) ~90°; (6) ~105°; (7) ~120°. Ordered; weight 0.25.
64. Shape of columella on inner margin [can differ from shape on outer margin, so the two coded separately]: (1) arching like half-circle; (2) arching in obtuse curve; (3) trending toward straight; (4) curving slightly into the aperture. Ordered; weight 0.5.
65. Outer margin shape: (1) more obtuse than inner margin; (2) same as inner margin; (3) more acute than inner margin. Weight 1.
66. Ontogenetic change in margin shape: (1) none; (2) becoming rounder over ontogeny. Weight 1.
67. Columella attitude [describing main trend of columella relative to coiling axis]: (1) 0° (*i. e.* perpendicular to coiling axis); (2) 15°; (3) 30°; (4) 45°; (5) 60°. Ordered; weight 0.25.
68. Columella lira [carina in middle of columella, visible in umbilicus]: (1) absent; (2) present. Weight 1.
69. Parietal inductura thickness [silicification can occur differently among different shell layers, which can affect characters such as this; therefore, "relative" states were coded based on comparisons among taxa found from the same beds, with comparisons among conspecifics from different beds used to establish the final character code]: (1) absent; (2) thinner than rest of shell; (3) thickness same as shell. Unordered; weight 1.
70. Columella reflected around coiling axis: (1) absent; (2) present. Weight 1.
71. Whole aperture inclined (tangential): (1) absent; (2) present. Weight 1.
72. Degree of whole aperture inclination (InAn): (1) ~10°; (2) ~20°; (3) ~30°; (4) ~40°; (5) ~50°; (6) ~60°. Ordered; weight 0.2.
73. Left side of aperture only inclined [or inclined at different angle than right side]: (1) absent; (2) present. Weight 1.
74. Left InAn: (1) ~10°; (2) ~20°; (3) ~30°. Ordered; weight 0.5.
75. Right side of aperture only inclined [or inclined at different angle than left side]: (1) absent; (2) present. Weight 1.
76. Right InAn: (1) ~10°; (2) ~20°; (3) ~30°. Ordered; weight 0.5.
77. Anterior projection of aperture [best observed from base, because growth lines will project forward instead of radially; it is important that they do this at the onset, as tangential aperture will cause growth lines on the periphery to slope forward as well]: (1) absent; (2) present. Weight 1.
78. Degree of anterior projection [relative to plane passing radially through coiling axis]: (1) 10°; (2) 20°; (3) 30°; (4) 40°; (5) 50°. Ordered; weight 0.25.
79. Aperture expansion [expansion of shell in radians as a "tube"]: (2) 0.05-0.10; (3) 0.10-0.15; (4) 0.15-0.20. Ordered; weight 0.5.
80. Curvature about coiling axis [in radians]: (3) $0.65 < K < 0.75$; (4) $0.75 < K < 0.85$; (5) $0.85 < K < 0.95$. Ordered; weight 0.5.
81. Translation (T) [vector of downwards growth (in radians)]: (5) $0.16 < T < 0.47$ (low dextral); (6) $0.47 < T < 0.79$ (moderate dextral); (7) $0.79 < T < 1.10$ (high dextral); (8) $1.10 < T < 1.41$ (very high dextral). Ordered; weight 0.33.
82. Continuous ontogenetic change in T: (2) isometric; (3) continuously increasing, resulting in ever-increasing apical angle. Weight 1.
83. Early ontogenetic change in T: (1) early decrease of T (*e. g. Trochonema, Trochonemella*); (2) isometric; (3) early increase in T. Unordered; weight 1.

84. Late ontogenetic change in T [often resulting in open-coiled "corkscrew"-like shells, *e. g. Lophospira helicteres, Eunema strigillata*]: (2) isometric; (3) late increase in T. Weight 1.
85. Punctuated late ontogenetic change in T: (1) occuring over 1-2 whorls (*e. g. Trochonema, Trochonemella*); (2) punctuated, occuring over less than one revolution. Weight 1.
86. Magnitude of late ontogenetic change in T: (1) slight, changing suture point; (2) major, resulting in open coiling. Weight 1.
87. Ornament on left side of aperture: (1) absent; (2) present on left ramp and base; (3) present on left ramp only. Unordered; weight 0.5.
88. LR ornament density [based on average angular distance between threads]: (1) 1 per 20°; (2) 1 per 10°; (3) 1 per 5°; (4) 1 per 1°. Ordered; weight 0.5.
89. LR ornament type: (1) thin local thickenings, little stronger than growth lines and with no profile (*e. g. Ruedemannia*); (2) lirae with weak profile (*e. g. Arjamannia*); (3) thick lirae with strong profile (*e. g. Longstaffia*). Weight 1.
90. RR ornament [because species with left ramp ornament sometimes lack right ramp ornament, these characters coded separately; completely inornate to completely ornate represents one step]: (1) absent; (2) present. Weight 0.5.
91. RR ornament density [see character 88]: (1) 1 per 20°; (2) 1 per 10°; (3) 1 per 5°; (4) 1 per 1°. Ordered; weight 0.5.
92. RR ornament strength: (1) thin threads; (2) weak lirae; (3) strong lirae. Ordered; weight 0.5.
93. Ornament as changes in aperture shape: (1) simple local thickenings of shell; (2) representing local flarings of aperture. Weight 1.
94. Ontogenetic change in RR ornament: (1) constant; (2) weaker on adult whorls. Weight 1.
95. Size: (1) <10 mm^3 (micro-mollusk); (2) small (>10 to <10^2 mm^3); (3) moderate (>10^2 to <10^3 mm^3); (4) large (>10^3 to <10^4 mm^3). Ordered; weight 0.33.

APPENDIX 2
CHARACTER MATRIX

Character data for lophospiroid species. See Appendix 1 for characters and character descriptions. [?, characters that could not be observed; -, characters that do not pertain to a species (*e. g.* ornament type for an inornate species); A-D denote polymorphic species [A, states 1 + 2; B, states 1 + 3; C, states 2 + 3; D, states 6 + 7]; BMNH, The Natural History Museum, London].

	Species	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
0	*Hormotoma simulatrix* (Billings, 1865)	2	5	3	3	1	1	2	1	4	2	2	3	1	-	?
0	*Ectomaria adelina* (Billings, 1865)	2	5	3	3	1	1	2	1	6	2	2	3	1	-	?
1	*Pagodospira cicelia* (Billings, 1865)	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
2	*Lophospira perangulata* (Hall, 1847)	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
3	*L. sororcula* (Billings, 1865)	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
4	*L. rectistriata* (Billings, 1865)	2	4	2	3	1	1	2	1	2	2	2	3	2	2	2
5	*Pagodospira derwiduii* Grabau, 1922	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
6	*Lophospira milleri* (Hall in Miller, 1889)	2	4	2	3	1	1	3	1	2	2	2	3	2	2	2
7	*Trochonemella knoxvillensis* Ulrich in Ulrich and Scofield, 1897	2	5	3	3	1	1	4	1	2	2	2	3	2	2	2
8	*Proturritella bicarinata* (Koken, 1889)	2	4	2	3	1	1	3	1	2	1	-	-	2	2	3
9	*Pagodospira dorothea* Grabau, 1922	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
10	*Trochonemella trochonemoides* (Ulrich in Ulrich and Scofield, 1897)	2	5	2	3	1	2	3	1	2	1	-	-	2	2	3
11	*Lophospira centralis* Ulrich and Scofield, 1897	2	3	2	2	1	1	3	1	2	2	2	3	2	2	2
12	*Trochonema bellula* Ulrich and Scofield, 1897	2	4	2	3	1	2	3	1	1	1	-	-	2	2	3
13	*T. eccentrica* Ulrich and Scofield, 1897	2	3	2	2	1	2	3	1	1	1	-	-	2	2	3
14	*Trochonemella montrealensis* Okulitch, 1935	2	4	1	3	1	2	4	1	2	2	2	3	1	-	-
15	*Lophospira helicteres* (Salter, 1859)	2	3	2	2	1	1	3	1	2	2	2	3	2	2	3
16	*Trochonemella* n. sp.	2	4	1	3	1	2	4	1	2	2	2	3	1	-	-
17	*Lophospira serrulata* (Salter, 1859)	2	5	3	3	1	1	4	1	2	2	2	3	2	2	3
18	*Eunema strigillata* Salter, 1859	2	3	2	2	1	1	3	1	1	1	-	-	2	2	3
19	*Trochonema umbilicata* (Hall, 1847)	2	3	2	2	1	2	3	1	1	1	-	-	2	2	3
20	*T. canadensis* Wilson, 1951	2	3	1	2	1	2	3	1	1	1	-	-	2	2	3
21	*T. wilsoni* Steele and Sinclair, 1971	2	3	2	2	1	2	3	1	1	1	-	-	2	2	3
22	*Lophospira ventricosa* (Salter, 1859)	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
23	*Trochonemella notablis* (Ulrich in Ulrich and Scofield, 1897)	2	4	1	3	1	2	4	1	2	2	2	3	1	-	-
24	*Gyronema pulchella* Ulrich and Scofield, 1897	2	3	1	2	1	1	2	1	1	1	-	-	2	2	2
25	*G. semicarinata* (Ulrich and Scofield, 1897)	2	3	1	2	1	1	2	1	1	1	-	-	2	2	2
26	*G. liljevalli* Rohr, 1980	2	3	2	2	1	2	3	1	1	1	-	-	2	2	3
27	*Trochonema madisonense* Ulrich and Scofield, 1897	2	4	2	3	1	2	3	1	1	1	-	-	2	2	3
28	*Lophospira burginensis* Ulrich and Scofield, 1897	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
29	*L. oweni* Ulrich and Scofield, 1897	2	5	3	3	1	1	2	1	2	2	2	3	2	2	1
30	*L. concinula* Ulrich and Scofield, 1897	2	3	1	2	1	1	3	1	2	2	2	3	2	2	3
31	*L. spironema* Ulrich and Scofield, 1897	2	4	2	3	1	1	3	1	2	2	2	3	2	2	2
32	*Donaldiella decursa* (Ulrich and Scofield, 1897)	2	5	3	3	1	1	2	1	2	2	1	3	2	2	2
33	*D. conoidea* (Ulrich and Scofield, 1897)	2	5	3	3	1	1	2	1	2	2	1	3	2	2	1
34	*D. producta* (Ulrich and Scofield, 1897)	2	5	3	3	1	1	2	1	2	2	1	2	2	2	2
35	*D.* Curdsville sp.	2	5	3	3	1	1	2	1	2	2	1	2	2	2	2
36	*Lophospira sumnerensis* (Safford, 1869)	2	5	3	3	1	1	2	1	2	2	2	3	2	2	1
37	*Trochonema salteri* Ulrich and Scofield, 1897	2	3	2	2	1	2	3	1	1	1	-	-	2	2	3
38	*Ruedemannia humilis* (Ulrich and Scofield, 1897)	2	4	2	3	1	1	2	1	4	2	2	2	2	1	2
39	*R. lirata* (Ulrich and Scofield, 1897)	2	4	2	3	1	1	2	1	4	2	2	2	2	1	2
40	*Lophospira tenuistriata* Ulrich and Scofield, 1897	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2

(continued)

APPENDIX 2. (continued)

41 *L. tropidophora* Meek, 1873	2	5	3	3	1	1	2	1	2	2	2	2	2	2	1
42 *Donaldiella filosa* (Donald, 1902)	2	5	3	3	1	1	2	1	2	2	2	3	2	2	2
43 *D. bowdeni* (Safford, 1869)	2	5	3	3	1	1	2	1	3	2	2	3	2	2	2
44 *Lophospira bellicarinata* Donald, 1906	2	4	1	3	1	1	3	1	2	2	2	3	2	2	4
45 *L. quadrisulcata* Ulrich and Scofield, 1897	2	3	1	2	1	1	3	1	2	2	2	3	2	2	3
46 "*Schizolopha*" *moorei* Ulrich and Scofield, 1897	2	5	3	3	1	1	4	1	2	2	2	4	2	2	1
47 *Lophospira* aff. *serrulata* [in Rohr, 1988]	2	4	1	3	1	1	4	1	2	2	2	3	2	2	3
48 *Trochonemella* BMNH 36364	2	4	1	3	1	1	4	1	2	2	2	3	2	2	3
49 *T. churkini* Rohr, 1988	2	4	2	3	1	2	4	1	2	2	2	3	1	-	-
50 *Trochonemella reusingi* Rohr and Blodgett, 1985	2	4	2	3	1	2	4	1	2	2	2	3	1	-	-
51 *Trochonema* aff. *umbilicata* [in Rohr, 1988]	2	2	2	2	1	2	3	1	1	1	-	-	2	2	3
52 *Arjamannia thraivensis* (Longstaff, 1924)	2	4	1	3	1	1	3	1	2	2	2	3	2	2	4
53 *Globonema bicarinata* (Wenz, 1938)	2	1	1	2	1	1	3	1	1	1	-	-	2	2	4
54 *Lophospira ?borkholmensis* (Koken, 1925)	2	4	2	3	1	1	2	1	4	2	2	2	2	1	2
55 *Trochonema pandori* Koken, 1925	2	4	2	3	1	2	3	1	1	1	-	-	2	2	3
56 *T.* aff. *pandori* Koken, 1925	2	4	2	3	1	2	3	1	1	1	-	-	2	2	3
57 *Lophospira sedgewicki* Donald, 1905	2	4	1	3	1	1	3	1	2	2	2	3	2	2	4
58 *L. ?hyaecinthinsis* Foerste, 1924	2	4	1	3	1	1	3	1	2	2	2	3	2	2	4
59 *Longstaffia centervillensis* (Foerste, 1923)	2	4	1	3	1	1	4	1	2	2	2	2	2	2	4
60 *Arjamannia cancellata* (M'Coy in Sedgwick and M'Coy, 1852)	2	4	1	3	1	1	3	1	2	2	2	2	2	2	4
61 *A. woodlandi* (Longstaff, 1924)	2	4	1	3	1	1	3	1	2	2	2	2	2	2	4
62 *Donaldiella trilineata* (Foerste, 1923)	2	5	3	3	1	1	2	1	3	2	2	3	2	2	1
63 *Lophospira gotlandica* Ulrich in Ulrich and Scofield, 1897	2	4	1	3	1	1	3	1	2	2	2	3	2	2	4
64 *Longstaffia laquetta* (Lindström, 1884)	2	4	1	3	2	1	4	2	2	2	2	2	2	1	4
65 *Arjamannia inexpectans* (Hall and Whitfield, 1872)	2	4	1	3	1	1	3	1	2	2	2	2	2	2	4
66 *Lophospira holmi* (Lindström, 1884)	2	4	1	3	1	1	3	1	2	2	2	2	2	2	4
67 *Kiviasukkaan nelsonae* Peel, 1975b	2	4	1	3	1	1	3	1	2	2	2	3	2	2	3
68 *Lophospira munda* (Lindström, 1884)	2	4	1	3	2	1	3	3	2	2	2	3	2	2	3
69 *L. imbricata* (Lindström, 1884)	2	4	1	3	2	1	4	4	2	2	2	3	2	2	3
70 *Trochonema turrita* (Lindström, 1884)	1	-	-	-	2	1	4	1	1	1	-	-	2	2	3
71 *Eunema kayesi* Rohr, 1981	1	-	-	-	2	1	4	1	1	1	-	-	2	2	3
72 *Ruedemannia laevissima* (Lindström, 1884)	2	4	2	3	1	1	2	1	4	2	2	2	2	1	1
73 *Arjamannia aulangonensis* (Peel, 1975a)	2	4	1	3	1	1	3	1	3	2	2	2	2	2	4
74 *Ruedemannia robusta* (Lindström, 1884)	2	4	2	3	1	1	2	1	4	2	2	2	2	1	1
75 *Longstaffia tubulosa* (Lindström, 1884)	2	4	1	3	2	1	4	3	2	2	2	2	2	1	4
76 *Longstaffia cyclonema* (Salter, 1873)	2	4	1	3	1	1	4	1	2	2	2	2	2	1	4
77 *Trochonema fatua* (Whiteaves, 1895)	2	1	2	2	1	1	3	1	1	1	-	-	2	2	3
78 *Loxoplocus soluta* (Whiteaves, 1884)	2	4	1	3	1	1	3	1	2	2	2	3	2	2	2
79 *Lophospira cochleata* (Lindström, 1884)	2	4	1	3	2	1	4	3	2	2	2	3	2	2	3
80 *Eunema muricata* (Lindström, 1884)	1	-	-	-	2	1	4	1	1	1	-	-	2	2	3
81 *Ruedemannia subrobusta* (Perner, 1907)	2	4	2	3	1	1	2	1	4	2	2	2	2	1	1
82 *Ptychozone aberrans* Perner, 1907	2	4	2	3	1	1	2	1	5	2	2	2	2	1	1

Species	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
0 *H. simulatrix*	?	2	1	-	-	1	2	-	-	1	1	1	-	3	2	1	2	5	4	2	6	6	2	1	1	-	-	-	-	?	?	?	1	2	2	1	-	-	?	1
0 *E. adelina*	?	2	1	-	-	1	1	-	-	1	1	1	-	3	2	5	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	1	1	1	1
1 *P. cicelia*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	5	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	A	1	1	1
2 *L. perangulata*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	5	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	A	1	1	1
3 *L. sorrorcula*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	4	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	1	1	1	1
4 *L. rectistriata*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	4	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	1	1	1	1
5 *P. derwiduii*	1	3	1	-	-	1	2	-	-	-	1	2	1	3	3	5	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	1	-	-	1	1
6 *L. milleri*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	4	3	3	4	1	5	3	1	-	2	2	3	1	1	1	1	1	1	3	1	2	2	2	1	1
7 *T. knoxvillensis*	1	2	1	-	-	1	2	-	-	-	1	2	2	6	3	5	2	3	4	1	5	3	1	-	2	2	3	1	1	1	1	1	1	3	2	2	A	2	1	1
8 *P. bicarinata*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	4	3	3	4	1	5	3	1	-	2	2	3	1	1	1	1	1	1	3	1	2	2	2	1	1
9 *P. dorothea*	1	3	1	-	-	1	2	-	-	-	1	2	1	3	3	3	2	3	3	1	5	3	1	-	2	1	1	1	1	1	1	1	1	3	2	1	-	-	1	1
10 *T. trochonemoides*	1	2	1	-	-	1	2	-	-	-	1	2	2	6	2	3	2	5	4	1	7	6	1	-	2	2	3	1	1	1	1	1	1	3	1	2	A	1	1	1
11 *L. centralis*	1	2	1	-	-	4	2	-	-	-	1	2	1	3	2	3	3	3	4	1	5	3	1	-	2	2	3	1	1	1	1	1	1	3	1	2	2	2	1	1
12 *T. bellula*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	5	2	2	2	2	3	1	2	2	1	1	1	3	1	2	2	2	1	1
13 *T. eccentrica*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	5	2	2	2	2	3	1	2	2	1	1	1	3	1	2	2	2	1	1
14 *T. montrealensis*	1	2	1	-	-	1	2	-	-	1	1	2	2	7	2	3	2	7	4	1	7	6	2	1	2	2	3	1	1	1	1	1	1	3	1	2	2	3	1	1
15 *L. helicteres*	1	2	1	-	-	4	2	-	-	-	1	2	1	3	2	3	3	3	4	1	5	3	2	1	2	3	3	1	2	1	1	1	1	3	1	2	2	2	1	1
16 *T.* n. sp.	1	2	2	1	2	1	3	1	1	1	1	2	2	7	1	2	2	7	7	1	7	6	2	1	2	3	3	1	1	2	2	1	1	3	1	2	2	3	1	1
17 *L. serrulata*	2	2	1	-	-	1	?	2	2	-	1	2	1	3	3	5	2	3	3	1	5	3	2	1	2	3	3	1	1	1	1	1	1	3	1	2	2	2	1	1
18 *E. strigillata*	1	2	1	-	-	4	2	-	-	-	1	2	2	4	2	3	3	3	4	1	5	3	2	1	2	3	3	1	2	1	1	1	1	3	1	2	2	2	1	1
19 *T. umbilicata*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	5	2	2	2	2	3	1	2	2	1	1	1	3	1	2	2	2	1	1
20 *T. canadensis*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	5	2	2	2	2	3	1	2	2	1	1	1	3	1	2	2	2	1	1
21 *T. wilsoni*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	6	2	2	2	2	3	1	2	2	1	1	1	3	1	2	2	2	1	1
22 *L. ventricosa*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	2	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	1	-	-	1	1
23 *T. notablis*	1	2	2	1	1	1	3	1	2	1	1	2	2	7	1	2	2	7	7	1	7	6	2	1	2	3	3	1	1	2	2	1	1	3	1	2	2	3	1	1
24 *G. pulchella*	1	2	1	-	-	4	2	-	-	-	1	2	1	3	2	3	3	3	4	1	5	3	1	-	2	2	3	1	1	1	1	1	1	3	1	2	2	2	1	2

(continued)

APPENDIX 2. (continued)

25 *G. semicarinata*	1	2	1	-	-	4	2	-	-	-	1	2	1	3	2	3	3	3	4	1	5	3	1	-	2	2	3	1	1	2	1	1	1	3	1	2	2	2	1	2
26 *G. liljevalli*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	1	2	2	7	7	1	7	5	2	2	2	2	3	1	2	2	1	1	1	3	1	2	2	2	1	2
27 *T. madisonense*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	5	2	2	2	3	3	1	2	2	1	1	1	3	1	2	2	2	1	1
28 *L. burginensis*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	3	2	3	3	1	5	3	1	-	2	1	1	1	1	1	1	1	1	2	2	2	1	1	1	1
29 *L. oweni*	1	3	1	-	-	1	2	-	-	2	1	2	1	3	3	3	2	3	3	1	5	3	1	-	2	1	1	2	1	1	1	1	1	2	2	2	1	1	1	1
30 *L. concinula*	1	2	1	-	-	4	2	-	-	-	1	2	1	3	2	3	3	3	4	1	5	3	2	1	2	3	3	1	2	1	1	1	1	3	1	2	2	2	1	1
31 *L. spironema*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	4	3	3	4	1	5	3	1	-	2	2	3	1	1	1	1	1	1	3	1	2	2	2	1	1
32 *D. decursa*	1	2	1	-	-	1	2	-	-	-	1	2	1	2	1	5	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	1	1	1	1
33 *D. conoidea*	1	2	1	-	-	1	2	-	-	-	1	2	1	2	2	3	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	1	1	1	1
34 *D. producta*	1	2	1	-	-	1	2	-	-	-	1	2	1	2	1	4	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	3	2	2	1	1	1	1
35 *D.* Curdsville sp.	1	2	1	-	-	1	2	-	-	-	1	2	1	2	1	4	2	3	3	1	5	3	1	-	2	1	1	1	1	1	1	1	1	3	2	2	1	1	1	1
36 *L. sumnerensis*	1	3	1	-	-	1	2	-	-	2	1	2	1	6	3	5	1	5	3	1	7	4	2	2	2	1	1	2	1	1	1	1	1	2	2	1	-	-	-	-
37 *T. salteri*	1	2	1	-	-	1	2	-	-	-	1	2	2	4	2	3	1	5	4	1	6	5	2	2	1	-	-	-	-	?	?	?	1	3	2	1	-	-	-	-
38 *R. humilis*	1	1	1	-	-	1	2	-	-	-	1	2	1	3	1	2	1	5	4	1	5	3	1	-	2	2	3	1	1	2	1	1	1	2	?	2	2	1	1	1
39 *R. lirata*	1	1	1	-	-	1	2	-	-	-	1	2	1	3	1	2	1	5	4	1	5	3	1	-	2	2	3	1	1	2	1	1	1	2	?	2	2	1	1	1
40 *L. tennuistriata*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	3	3	2	3	3	1	5	3	1	-	2	1	3	1	1	1	1	1	1	2	2	2	1	1	1	1
41 *L. tropidophora*	1	3	1	-	-	1	2	-	-	2	1	2	1	3	3	3	2	3	3	1	5	3	1	-	2	1	1	2	1	1	1	1	1	2	2	2	1	1	1	1
42 *D. filosa*	1	3	1	-	-	1	2	-	-	-	1	2	1	3	3	5	2	3	3	1	5	3	1	-	1	1	1	1	1	1	1	1	1	2	2	1	-	-	-	-
43 *D. bowdeni*	1	3	1	-	-	1	2	-	-	2	1	2	1	3	3	3	2	3	3	1	5	3	1	-	2	1	1	2	1	1	1	1	1	2	2	2	1	1	1	1
44 *L. bellicarinata*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	3	5	3	3	7	1	6	4	2	2	2	2	3	1	1	2	1	1	2	3	1	2	1	2	1	1
45 *L. quadrisulcata*	1	2	1	-	-	4	2	-	-	-	1	2	1	3	3	4	3	3	4	1	5	3	2	1	2	3	3	1	2	1	1	1	1	3	1	2	2	2	1	2
46 *L. moorei*	1	3	2	2	1	4	2	-	-	2	1	2	1	3	3	3	2	3	3	1	5	3	1	-	1	-	-	-	-	?	?	?	1	2	2	2	1	1	1	-
47 *L.* aff. *L. serrulata*	?	2	2	1	1	1	3	2	2	-	1	2	1	5	1	4	2	7	7	1	7	6	1	-	2	3	3	1	1	2	2	2	1	3	1	2	2	3	2	1
48 *T.* BMNH 36364	?	2	2	1	1	1	3	2	2	-	1	2	1	5	1	4	2	7	7	1	7	6	1	-	2	3	3	1	1	2	2	2	1	3	1	2	2	3	2	2
49 *T. churkini*	1	2	2	1	1	1	3	1	2	1	1	2	2	6	1	2	2	7	7	1	7	6	1	-	2	3	3	1	1	2	2	2	1	3	1	2	2	3	1	1
50 *T. reusingi*	1	2	2	1	1	1	3	1	2	1	1	2	2	6	1	2	2	7	7	1	7	6	1	-	2	3	3	1	1	2	2	2	1	3	1	2	2	3	2	1
51 *T.* aff. *umbilicata*	1	2	1	-	-	1	2	-	-	-	1	2	2	4	2	3	2	7	7	1	6	5	2	2	2	2	3	1	2	2	1	1	1	3	1	2	2	3	1	1
52 *A. thraivensis*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	3	4	3	3	7	1	6	4	2	2	2	2	3	1	1	2	1	1	2	3	1	2	1	2	1	1
53 *G. bicarinata*	1	3	1	-	-	1	2	-	-	-	1	2	2	4	2	3	1	5	4	2	6	6	2	2	1	-	-	-	-	?	?	?	1	3	2	1	-	-	-	-
54 *L. ?borkholmensis*	1	1	1	-	-	1	2	-	-	-	1	2	1	3	1	2	1	5	4	1	5	3	1	-	1	-	-	-	-	?	?	?	1	2	?	2	2	1	1	1
55 *T. pandori*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	5	2	2	2	3	3	1	2	2	1	1	1	3	1	2	2	2	1	1
56 *T.* aff. *pandori*	1	2	1	-	-	1	2	-	-	-	1	2	2	7	2	3	2	7	7	1	7	5	2	1	2	3	3	1	2	1	1	1	1	3	1	2	2	2	1	1
57 *L. sedgewicki*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	3	5	3	5	7	1	6	4	2	2	1	-	-	-	-	?	?	?	2	2	1	2	1	1	1	1
58 *L. ?hyaecinthinsis*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	3	5	3	5	7	1	6	4	2	2	1	-	-	-	-	?	?	?	2	2	1	2	1	1	1	1
59 *L. centervillensis*	1	3	1	-	-	1	2	-	-	-	2	2	1	3	3	3	3	4	6	1	6	4	1	-	2	2	3	1	1	2	1	1	1	1	1	2	2	2	1	1
60 *A. cancellata*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	3	3	3	3	7	1	6	4	2	2	2	2	3	1	1	2	1	1	2	3	1	2	1	1	1	1
61 *A. woodlandi*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	2	3	3	3	7	1	6	4	2	2	?	?	?	?	1	1	1	1	2	3	1	2	1	1	1	1
62 *D. trilineata*	1	3	1	-	-	1	2	-	-	-	1	2	1	3	3	3	2	3	3	1	5	3	1	-	?	-	-	-	-	?	?	?	1	3	2	1	-	-	?	?
63 *L. gotlandica*	1	3	1	-	-	4	2	-	-	-	2	2	1	6	3	5	3	5	7	1	6	4	1	-	1	-	-	-	-	?	?	?	2	2	?	1	-	-	-	-
64 *L. laquetta*	1	2	1	-	-	1	2	-	-	-	2	2	1	3	1	2	3	4	6	1	6	5	1	-	2	2	3	1	1	2	1	1	1	1	1	2	2	2	1	1
65 *A. inexpectans*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	2	3	3	3	7	1	6	4	2	2	2	1	2	2	1	2	1	1	2	3	1	2	1	1	1	1
66 *L. holmi*	1	3	1	-	-	1	2	-	-	-	2	2	1	3	3	2	1	5	4	1	5	3	1	-	2	2	3	1	1	2	1	1	1	2	1	1	-	-	-	-
67 *K. nelsonae*	1	3	1	-	-	4	2	-	-	-	2	2	1	6	3	5	3	5	7	1	6	4	1	-	1	-	-	-	-	?	?	?	2	2	?	1	-	-	-	-
68 *L. munda*	2	3	1	-	-	1	3	2	2	-	2	2	1	4	3	3	1	5	3	1	6	4	2	2	1	-	-	-	-	?	?	?	2	3	1	2	2	2	1	1
69 *L. imbricata*	2	3	1	-	-	1	3	2	2	-	2	2	1	4	2	3	1	5	3	1	6	4	2	2	1	-	-	-	-	?	?	?	2	3	1	2	2	2	1	1
70 *T. turrita*	1	3	1	-	-	1	2	-	-	-	1	2	2	3	1	2	2	7	7	2	7	7	2	2	2	2	3	1	1	2	1	1	1	2	1	2	2	2	1	1
71 *E. kayesi*	1	3	1	-	-	1	2	-	-	-	1	2	2	3	1	2	2	7	7	2	7	7	2	2	2	2	3	1	1	2	1	1	1	2	1	2	2	2	1	1
72 *R. laevissima*	1	2	1	-	-	1	2	-	-	-	1	2	1	3	1	2	1	5	4	1	5	3	1	-	1	-	-	-	-	?	?	?	1	1	?	2	2	1	?	?
73 *A. aulangonensis*	1	3	1	-	-	1	2	-	-	-	2	2	1	4	2	3	3	3	7	1	6	4	1	-	2	1	2	2	1	2	1	1	2	3	1	?	?	?	?	?
74 *R. robusta*	1	1	1	-	-	1	2	-	-	-	1	2	1	3	1	2	1	5	4	1	5	3	1	-	2	1	3	1	1	2	1	1	1	1	?	2	2	1	?	?
75 *L. tubulosa*	2	3	1	-	-	1	3	2	2	-	2	2	1	3	3	3	3	3	7	1	6	5	1	-	2	2	3	1	1	2	1	1	2	1	1	2	2	2	1	2
76 *L. cyclonema*	1	3	1	-	-	1	2	-	-	-	2	2	1	3	3	2	3	4	6	1	6	4	1	-	2	2	3	1	1	2	1	1	1	1	1	2	2	2	1	1
77 *T. fatua*	1	2	1	-	-	1	2	-	-	-	1	2	2	4	2	3	1	5	4	2	6	6	2	2	1	-	-	-	-	?	?	?	1	3	2	1	-	-	-	-
78 *L. soluta*	1	3	1	-	-	?	2	-	-	-	2	2	1	6	3	5	1	5	7	1	6	4	1	-	1	-	-	-	-	?	?	?	2	2	?	1	-	-	-	-
79 *L. cochleata*	1	3	1	-	-	4	3	2	2	-	2	2	1	4	3	5	1	5	3	1	6	4	2	2	1	-	-	-	-	?	?	?	2	3	1	2	2	2	1	1
80 *E. muricata*	1	3	1	-	-	1	2	-	-	-	1	2	2	3	1	2	2	7	7	2	7	7	2	2	2	2	3	1	1	2	1	1	1	2	1	2	2	2	1	1
81 *R. subrobusta*	1	1	1	-	-	1	2	-	-	-	1	2	1	3	2	2	1	5	4	1	5	3	1	-	2	1	3	1	1	2	1	1	1	1	?	2	2	1	?	?
82 *P. aberrans*	1	1	1	-	-	1	2	-	-	-	1	1	-	3	2	1	1	5	4	1	5	3	1	-	1	-	-	-	-	?	?	?	1	0	?	2	2	1	?	?

Species	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
0 *H. simulatrix*	-	2	1	-	-	-	?	2	3	2	1	2	1	?	1	1	-	1	-	1	-	1	-	2	5	8	3	2	2	-	1	1	-	-	1	-	-	-	-	2
0 *E. adelina*	1	2	1	-	-	-	?	2	3	2	1	1	1	3	2	1	-	1	-	1	-	1	-	2	5	8	3	2	2	-	1	1	-	-	1	-	-	-	-	1
1 *P. cicelia*	1	2	1	-	-	-	?	2	3	2	1	1	1	3	2	1	-	1	-	1	-	1	-	2	5	8	3	2	2	-	1	1	-	-	1	-	-	-	-	1
2 *L. perangulata*	1	2	1	-	-	-	?	2	3	2	1	1	1	3	2	1	-	1	-	1	-	1	-	2	5	7	3	2	C	1	1	1	-	-	1	-	-	-	-	1
3 *L. sorrorcula*	1	2	1	-	-	-	?	3	3	2	1	1	1	3	2	1	-	1	-	1	-	1	-	3	6	6	3	2	2	-	1	1	-	-	1	-	-	-	-	2
4 *L. rectistriata*	1	2	1	-	-	-	?	3	3	2	1	1	1	3	2	1	-	1	-	1	-	1	-	3	6	6	3	2	2	-	-	1	-	-	1	-	-	-	-	2
5 *P. derwiduii*	-	?	1	-	-	-	?	2	3	2	1	1	1	3	?	1	-	1	-	1	-	1	-	2	5	8	3	2	C	1	1	1	-	-	1	-	-	-	-	1
6 *L. milleri*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	3	6	6	3	2	2	-	-	1	-	-	1	-	-	-	-	1
7 *T. knoxvillensis*	1	2	2	1	2	1	2	4	2	2	1	4	1	4	1	2	3	1	-	1	-	2	2	4	4	5	3	1	3	1	1	1	-	-	1	-	-	-	-	2
8 *P. bicarinata*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	3	6	6	3	2	2	-	-	2	3	3	2	4	3	1	1	1

(continued)

APPENDIX 2. (continued)

9 *P. dorothea*	-	?	1	-	-	-	?	2	3	2	1	1	1	3	?	1	-	1	-	1	-	1	-	2	5	8	3	2	2	-	-	1	-	-	1	-	-	-	-	1
10 *T. trochonemoides*	2	1	2	3	2	2	2	4	2	2	1	5	1	4	1	2	3	1	-	1	-	2	3	4	4	5	3	1	3	1	1	1	-	-	1	-	-	-	-	2
11 *L. centralis*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	2	3	1	-	1	-	3	6	6	3	2	2	-	-	1	-	-	1	-	-	-	-	1
12 *T. bellula*	2	1	2	3	2	1	2	5	2	2	1	5	1	4	1	2	5	1	-	1	-	2	3	4	4	5	3	1	3	1	1	1	-	-	1	-	-	-	-	2
13 *T. eccentrica*	2	1	2	3	2	1	2	5	2	2	1	5	2	4	1	2	6	1	-	1	-	2	4	4	4	5	3	1	3	-	1	1	-	-	1	-	-	-	-	2
14 *T. montrealensis*	2	2	2	1	1	1	1	4	2	2	1	4	1	4	1	2	3	1	-	1	-	2	3	4	4	5	3	1	3	1	1	1	-	-	1	-	-	-	-	2
15 *L. helicteres*	2	2	2	1	1	2	2	4	2	2	2	2	1	4	2	1	-	2	2	1	-	1	-	3	6	D	3	3	3	2	2	1	-	-	1	-	-	-	-	2
16 *T.* n. sp.	2	2	2	1	1	1	1	5	2	2	1	4	1	4	1	2	4	1	-	1	-	2	2	4	4	5	3	1	3	1	2	1	-	-	1	-	-	-	-	2
17 *L. serrulata*	1	2	2	1	1	1	2	2	3	2	1	1	1	3	2	1	-	1	-	1	-	1	-	2	5	D	3	3	3	2	2	1	-	-	1	-	-	-	-	1
18 *E. strigillata*	2	2	2	1	1	2	2	4	3	2	2	2	1	4	2	1	-	1	-	1	-	1	-	3	6	D	3	2	3	2	2	1	-	-	1	-	-	-	-	2
19 *T. umbilicata*	2	1	2	3	2	1	1	6	1	2	1	5	2	4	1	2	6	1	-	1	-	2	4	4	4	5	3	1	3	-	1	1	-	-	1	-	-	-	-	2
20 *T. canadensis*	2	1	2	3	2	1	1	6	1	2	1	5	2	4	1	2	6	1	-	1	-	2	4	4	4	5	3	1	3	-	1	1	-	-	1	-	-	-	-	2
21 *T. wilsoni*	2	1	2	3	2	1	1	6	1	2	1	5	2	4	1	2	6	1	-	1	-	2	4	4	4	5	3	1	3	-	1	1	-	-	1	-	-	-	-	2
22 *L. ventricosa*	-	2	1	-	-	-	?	2	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	3	5	7	3	3	2	-	1	1	-	-	1	-	-	-	-	1
23 *T. notablis*	2	2	2	1	1	1	1	5	2	2	1	3	1	4	1	2	4	1	-	1	-	2	4	4	4	5	3	1	3	1	2	1	-	-	1	-	-	-	-	2
24 *G. pulchella*	1	2	?	-	-	-	?	4	3	2	1	2	1	3	2	1	-	2	3	1	-	1	-	3	6	6	3	2	2	-	-	2	2	3	1	-	-	-	-	1
25 *G. semicarinata*	1	2	?	-	-	-	?	4	3	2	1	2	1	3	2	1	-	2	3	1	-	1	-	3	6	6	3	2	2	-	-	2	2	3	2	2	3	1	1	1
26 *G. liljevalli*	2	1	1	-	-	-	?	6	1	2	1	5	2	4	1	2	6	1	-	1	-	2	5	4	4	5	3	1	3	-	1	1	-	-	1	-	-	-	-	1
27 *T. madisonense*	2	1	2	3	2	1	2	6	2	2	1	5	1	4	1	2	6	1	-	1	-	2	3	4	4	5	3	1	3	-	1	1	-	-	1	-	-	-	-	3
28 *L. burginensis*	1	3	1	-	-	-	?	3	3	1	1	1	1	3	1	1	-	1	-	1	-	1	-	3	5	6	3	2	2	-	-	1	-	-	1	-	-	-	-	1
29 *L. oweni*	1	4	1	-	-	-	?	3	3	1	1	2	1	3	1	1	-	1	-	1	-	1	-	3	5	6	3	2	2	-	-	1	-	-	1	-	-	-	-	2
30 *L. concinula*	2	2	2	3	1	2	2	4	3	2	1	2	1	4	2	1	-	2	1	2	1	1	-	3	6	6	3	2	3	1	1	1	-	-	1	-	-	-	-	1
31 *L. spironema*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	3	6	6	3	2	2	-	-	2	4	1	2	4	1	1	1	1
32 *D. decursa*	1	2	1	-	-	-	?	2	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	2	5	7	3	2	2	-	1	1	-	-	1	-	-	-	-	1
33 *D. conoidea*	1	2	1	-	-	-	?	2	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	2	5	7	3	2	2	-	1	1	-	-	1	-	-	-	-	2
34 *D. producta*	1	2	1	-	-	-	?	2	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	2	5	8	3	2	3	1	1	1	-	-	1	-	-	-	-	2
35 *D.* Curdsville sp.	1	2	1	-	-	-	?	2	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	2	5	8	3	2	3	2	2	1	-	-	1	-	-	-	-	2
36 *L. sumnerensis*	-	4	1	-	-	-	?	3	3	1	1	2	1	3	1	2	3	1	-	1	-	1	-	3	6	7	3	2	2	-	-	1	-	-	1	-	-	-	-	2
37 *T. salteri*	-	1	2	3	2	1	1	6	1	2	1	3	1	4	1	1	-	1	-	1	-	2	1	3	5	6	3	2	2	-	-	1	-	-	1	-	-	-	-	2
38 *R. humilis*	1	2	1	-	-	-	?	4	2	2	1	2	1	3	2	1	-	1	-	1	-	1	-	4	6	6	2	2	2	-	-	1	-	-	1	-	-	-	-	1
39 *R. lirata*	1	2	1	-	-	-	?	4	2	2	1	2	1	3	2	1	-	1	-	1	-	1	-	4	6	6	2	2	2	-	-	2	4	1	1	-	-	-	-	1
40 *L. tennuistriata*	1	3	1	-	-	-	?	3	3	1	1	1	1	3	?	1	-	1	-	1	-	1	-	3	5	7	3	2	2	-	-	2	3	1	2	3	1	1	1	1
41 *L. tropidophora*	1	4	1	-	-	-	?	3	3	1	1	2	1	3	1	1	-	1	-	1	-	1	-	3	5	6	3	2	2	-	-	1	-	-	1	-	-	-	-	2
42 *D. filosa*	-	2	1	-	-	-	?	2	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	2	5	8	3	2	2	-	1	1	-	-	1	-	-	-	-	1
43 *D. bowdeni*	1	3	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	2	5	8	3	2	2	1	1	1	-	-	1	-	-	-	-	2
44 *L. bellicarinata*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	2	1	1	-	3	6	7	3	2	2	-	-	1	-	-	1	-	-	-	-	2
45 *L. quadrisulcata*	2	2	2	3	1	2	2	4	3	2	1	2	1	4	2	1	-	2	1	2	1	1	-	3	6	6	3	2	3	1	1	1	-	-	1	-	-	-	-	2
46 *L. moorei*	-	?	1	-	-	-	?	3	3	1	1	2	1	?	?	1	-	1	-	1	-	1	-	3	5	6	3	2	2	-	-	1	-	-	1	-	-	-	-	2
47 *L.* aff. *serrulata*	2	2	2	3	1	1	2	4	3	2	1	2	1	3	1	2	1	1	-	2	1	2	1	3	5	6	3	2	3	1	1	1	-	-	1	-	-	-	-	1
48 *T.* BMNH 36364	2	2	2	3	1	1	2	4	2	2	1	2	1	3	1	2	1	1	-	2	1	2	2	3	5	6	3	2	3	1	1	1	-	-	1	-	-	-	-	2
49 *T. churkini*	2	2	2	1	1	1	1	5	2	2	1	3	1	4	1	2	5	1	-	2	1	2	2	4	4	6	3	2	3	1	2	1	-	-	1	-	-	-	-	2
50 *T. reusingi*	2	2	2	1	1	1	1	5	2	2	1	3	1	4	1	2	5	1	-	2	1	2	2	4	4	6	3	2	3	1	2	1	-	-	1	-	-	-	-	2
51 *T.* aff. *umbilicata*	1	1	2	3	2	1	1	6	1	2	1	4	1	4	1	2	2	1	-	1	-	2	2	3	5	6	3	1	2	-	1	1	-	-	1	-	-	-	-	2
52 *A. thraivensis*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	2	2	1	-	3	6	7	3	2	2	1	1	2	3	3	1	-	-	-	-	2
53 *G. bicarinata*	-	1	2	2	3	1	2	6	3	2	1	3	1	4	1	1	-	1	-	1	-	2	1	3	6	6	3	2	3	-	1	1	-	-	1	-	-	-	-	2
54 *L. ?borkholmensis*	1	?	1	-	-	-	?	4	1	2	1	4	1	?	?	1	-	1	-	1	-	1	-	4	5	6	2	2	2	-	-	2	4	1	2	3	1	1	1	2
55 *T. pandori*	2	1	2	3	2	1	1	6	2	2	1	5	1	4	1	2	5	1	-	1	-	2	4	4	4	5	3	1	2	-	1	1	-	-	1	-	-	-	-	2
56 *T.* aff. *pandori*	2	1	2	3	2	1	1	6	2	2	1	5	1	4	1	2	3	1	-	1	-	2	4	4	4	6	3	1	3	1	1	1	-	-	1	-	-	-	-	2
57 *L. sedgewicki*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	2	1	1	-	3	6	7	3	2	3	1	1	1	-	-	1	-	-	-	-	1
58 *L. ?hyaecinthinsis*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	2	1	1	-	3	6	7	3	2	3	1	1	2	3	1	2	3	1	1	1	1
59 *L. centervillensis*	1	2	1	-	-	-	?	4	2	2	1	2	1	3	2	1	-	1	-	2	1	1	-	4	6	6	3	2	2	-	-	2	2	3	1	-	-	-	-	2
60 *A. cancellata*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	2	2	1	-	3	6	7	3	2	2	1	1	2	4	3	2	4	2	1	2	2
61 *A. woodlandi*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	2	2	1	-	3	6	6	3	2	2	1	1	2	4	3	2	4	2	1	1	2
62 *D. trilineata*	-	2	?	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	1	-	1	-	2	6	8	3	2	2	-	1	1	-	-	1	-	-	-	-	2
63 *L. gotlandica*	-	3	2	3	2	1	2	4	3	2	1	2	1	B	2	1	-	1	-	2	2	1	-	3	6	7	3	2	3	1	1	1	-	-	1	-	-	-	-	3
64 *L. laquetta*	1	2	1	-	-	-	?	4	2	2	1	2	1	3	2	1	-	1	-	2	1	1	-	4	6	6	3	2	2	-	-	2	2	3	1	-	-	-	-	2
65 *A. inexpectans*	1	2	1	-	-	-	?	4	3	2	1	2	1	3	2	1	-	1	-	2	2	1	-	3	6	6	3	2	2	1	1	2	4	3	2	4	2	1	1	2
66 *L. holmi*	-	2	1	-	-	-	?	4	2	2	1	2	1	3	2	1	-	1	-	2	2	1	-	4	6	6	3	2	2	-	-	2	4	1	2	4	1	1	1	1
67 *K. nelsonae*	-	3	2	3	3	1	2	4	3	2	1	2	1	3	2	1	-	1	-	2	3	1	-	3	6	7	3	2	3	2	2	1	-	-	1	-	-	-	-	3
68 *L. munda*	1	2	1	-	-	-	?	4	3	2	1	2	1	1	2	1	-	1	-	2	2	1	-	2	6	8	3	2	3	1	1	1	-	-	1	-	-	-	-	0
69 *L. imbricata*	1	2	1	-	-	-	?	4	3	2	1	2	1	1	2	1	-	1	-	2	2	1	-	2	6	8	3	2	3	1	1	2	3	3	2	3	2	1	1	0
70 *T. turrita*	2	1	2	2	3	1	1	5	2	2	1	3	1	4	1	1	-	1	-	1	-	2	1	3	6	7	3	2	3	1	1	2	3	3	1	-	-	-	-	2
71 *E. kayesi*	2	1	2	2	3	1	1	5	2	2	1	3	1	4	1	1	-	1	-	1	-	2	1	3	6	7	3	2	3	1	1	2	3	1	1	-	-	-	-	2
72 *R. laevissima*	1	1	1	-	-	-	?	4	2	2	1	2	1	1	2	1	-	1	-	1	-	1	-	4	6	6	2	2	2	-	-	2	4	1	1	4	1	-	1	2
73 *A. aulangonensis*	1	?	?	?	?	?	?	4	3	2	1	2	1	?	?	1	-	1	-	2	2	?	?	3	6	6	?	2	2	1	1	2	4	3	2	4	2	1	1	2
74 *R. robusta*	1	1	1	-	-	-	?	4	2	2	1	2	1	1	2	1	-	1	-	1	-	1	-	4	6	6	2	2	2	-	-	2	4	1	2	3	1	1	1	3
75 *L. tubulosa*	1	2	1	-	-	-	?	4	2	2	1	2	1	3	2	1	-	1	-	2	1	1	-	4	6	6	3	2	2	-	-	2	1	3	1	2	3	2	1	2
76 *L. cyclonema*	1	2	1	-	-	-	?	4	2	2	1	2	1	3	2	1	-	1	-	2	1	1	-	4	6	6	3	2	2	-	-	2	2	3	1	-	-	-	-	2
77 *T. fatua*	-	1	2	3	2	1	1	6	1	2	1	3	1	4	1	1	-	1	-	1	-	2	1	3	5	6	3	2	2	-	-	1	-	-	1	-	-	-	-	2
78 *L. soluta*	-	?	2	3	3	1	2	4	3	2	1	2	1	3	?	?	?	1	-	2	-	1	-	3	6	8	?	?	2	?	-	1	-	-	1	-	-	-	-	3
79 *L. cochleata*	1	3	1	-	-	-	?	4	3	2	1	2	1	1	2	1	-	1	-	2	2	1	-	2	6	8	3	2	3	1	1	2	3	1	2	3	1	1	1	0
80 *E. muricata*	2	1	2	2	3	1	1	5	2	2	1	3	1	4	1	1	-	1	-	1	-	2	1	3	6	6	3	2	3	1	1	1	-	-	1	-	-	-	-	2
81 *R. subrobusta*	1	1	1	-	-	-	?	4	2	2	1	2	1	1	2	1	-	1	-	1	-	1	-	4	6	6	2	2	2	-	-	2	4	1	2	2	1	1	1	3
82 *P. aberrans*	1	1	1	-	-	-	?	4	3	2	1	2	1	1	?	2	1	1	-	1	-	1	-	4	6	6	2	2	2	-	-	2	3	1	2	3	2	1	1	2

APPENDIX 3
STRATIGRAPHIC DATA

Range and sampling data for lophospiroid species. Separate horizon scales are used for each realm when calculating the likelihood of gaps (Wagner, 1995a). Stratigraphic debt and discrete ranges were calculated using the scales on Fig. 1, where Cassinian = Middle Arenig, Fennian = Late Arenig, Llanvirn = Early Llanvirn, Llandeilo = Late Llanvirn, Ashbian-Black Riveran = Early Caradoc, Rocklandian-Kirkfieldian = Middle1 Caradoc, Shermanian-Edenian = Middle2 Caradoc, Maysvillian-Richmondian = Ashgill, Rhuddanian-Aeronian = Early Llandovery, Telychian = Late Llandovery, Sheinwoodian = Early Wenlock, Homerian = Late Wenlock, Gorstian = Early Ludlow, and Ludfordian = Late Ludlow. [FKA, oldest sampled horizon, counted as number of older lophospiroid horizons known up to that point (see Wagner, 1995a); FSA, first stage from which a species is known; H, number of horizons from which a species is known; LKA, latest sampled horizons; LSA, last stage from which a species is known; Realm, biogeographic province: Baltica (Balt), Gondwana (Gond), Laurentia (Laur), Toquima-Tablehead (ToqTab)].

No.	Species	H	FKA	LKA	Realm	FSA	LSA
1	*Pagodospira cicelia*	14	1	57	Laur	Cassinian	Llanvirn
2	*Lophospira perangulata*	85	1	256	Laur	Cassinian	Richmondian
3	*L. sorrorcula*	16	18	57	ToqTab	Fennian	Llandeilo
4	*L. rectistriata*	6	18	44	Laur	Fennian	Llandeilo
5	*Pagodospira derwiduii*	7	18	27	ToqTab	Fennian	Llanvirn
6	*Lophospira milleri*	49	18	256	Laur	Llanvirn	Richmondian
7	*Trochonemella knoxvillensis*	7	18	44	Laur	Llanvirn	Llandeilan
8	*Proturritella bicarinata*	19	22	48	Balt	Llandeilan	Llandeilan
9	*Pagodospira dorothea*	1	22	44	ToqTab	Llandeilo	Llandeilan
10	*Trochonemella trochonemoides*	5	22	46	Laur	Llandeilo	Ashby
11	*Lophospira centralis*	35	45	220	Laur	Ashby	Rocklandian
12	*Trochonema bellula*	1	45	46	Laur	Ashby	Black Riveran
13	*T. eccentrica*	1	45	47	Laur	BlackRiveran	Black Riveran
14	*Trochonemella montrealensis*	8	47	91	Laur	BlackRiveran	Black Riveran
15	*Lophospira helicteres*	16	47	131	Laur	BlackRiveran	Rocklandian
16	*Trochonemella* n. sp.	3	47	91	Laur	BlackRiveran	Black Riveran
17	*Lophospira serrulata*	16	47	137	Laur	BlackRiveran	Kirkfieldian
18	*Eunema strigillata*	5	47	131	Laur	BlackRiveran	Black Riveran
19	*Trochonema umbilicata*	31	47	203	Laur	BlackRiveran	Shermanian
20	*T. canadensis*	3	47	91	Laur	BlackRiveran	Shermanian
21	*T. wilsoni*	1	47	58	Laur	BlackRiveran	Shermanian
22	*L. ventricosa*	13	59	208	Laur	BlackRiveran	Kirkfieldian
23	*Trochonemella notablis*	4	92	131	Laur	BlackRiveran	Black Riveran
24	*Gyronema semicarinata*	6	59	131	Laur	Rocklandian	Rocklandian
25	*G. pulchella*	4	92	137	Laur	BlackRiveran	Rocklandian
26	*G. liljevalli*	1	97	107	ToqTab	Rocklandian	Rocklandian
27	*Trochonema madisonense*	7	97	256	Laur	Kirkfieldian	Richmondian
28	*Lophospira burginensis*	13	97	209	Laur	Rocklandian	Edenian
29	*L. oweni*	11	119	209	Laur	Rocklandian	Edenian
30	*L. concinula*	4	132	165	Laur	Rocklandian	Rocklandian
31	*L. spironema*	2	132	137	Laur	Rocklandian	Rocklandian
32	*Donaldiella decursa*	1	155	184	Laur	Kirkfieldian	Shermanian
33	*D. conoidea*	1	155	165	Laur	Kirkfieldian	Shermanian
34	*D. producta*	1	155	165	Laur	Kirkfieldian	Shermanian
35	*D.* Curdsville sp.	1	187	208	Laur	Kirkfieldian	Shermanian
36	*Lophospira sumnerensis*	15	138	208	Laur	Shermanian	Richmondian
37	*Trochonema salteri*	8	138	265	Laur	Shermanian	Richmondian
38	*Ruedemannia humilis*	3	155	184	Laur	Edenian	Richmondian
39	*R. lirata*	6	187	253	Laur	Edenian	Richmondian
40	*Lophospira tenuistriata*	3	187	220	Laur	Edenian	Richmondian
41	*L. tropidophora*	29	187	256	Laur	Edenian	Richmondian
42	*D. filosa*	1	187	208	Laur	Edenian	Edenian
43	*D. bowdeni*	21	187	256	Laur	Edenian	Richmondian
44	*Lophospira bellicarinata*	2	210	256	Laur	Maysvillian	Richmondian
45	*L. quadrisulcata*	6	210	220	Laur	Maysvillian	Maysvillian
46	*L. moorei*	7	221	256	Laur	Richmondian	Richmondian
47	*L.* aff. *serrulata*	3	221	256	Laur	Richmondian	Richmondian
48	*Trochonemella* BMNH 36364	1	221	256	Laur	Richmondian	Richmondian
49	*T. churkini*	3	221	256	Laur	Richmondian	Richmondian

(continued)

APPENDIX 3. (continued)

50	*T. reusingi*	3	221	256	Laur	Richmondian	Richmondian
51	*Trochonema* aff. *umbilicata*	3	221	256	Laur	Richmondian	Richmondian
52	*Arjamannia sybellina*	5	221	256	Laur	Richmondian	Richmondian
53	*Globonema bicarinata*	2	223	239	Balt	Richmondian	Richmondian
54	*Lophospira ?borkholmensis*	2	223	239	Balt	Richmondian	Richmondian
55	*Trochonema pandori*	2	223	239	Balt	Richmondian	Richmondian
56	*T.* aff. *pandori*	1	223	239	Balt	Richmondian	Richmondian
57	*Lophospira sedgewicki*	5	223	273	Laur	Richmondian	Telychian
58	*L.?hyacinthensis*	2	223	256	Laur	Richmondian	Richmondian
59	*Longstaffia centervillensis*	3	257	273	Laur	Rhuddanian	Telychian
60	*Arjamannia cancellata*	1	257	261	Laur	Rhuddanian	Telychian
61	*A. woodlandi*	5	257	273	Laur	Rhuddanian	Telychian
62	*D. trilineata*	3	257	265	Laur	Aeronian	Aeronian
63	*Lophospira gotlandica*	30	257	337	Laur	Aeronian	Ludfordian
64	*Longstaffia laquetta*	6	257	301	Laur	Rhuddanian	Homerian
65	*A. inexpectans*	3	262	273	Laur	Telychian	Telychian
66	*Lophospira holmi*	1	275	277	Laur	Sheinwoodian	Sheinwoodian
67	*Kiviasukkaan nelsonae*	1	275	277	Laur	Sheinwoodian	Sheinwoodian
68	*Lophospira munda*	1	282	294	Laur	Sheinwoodian	Homerian
69	*L. imbricata*	7	278	330	Laur	Sheinwoodian	Gorstian
70	*Eunema turrita*	5	278	308	Laur	Sheinwoodian	Homerian
71	*E. kayesi*	1	282	294	Laur	Sheinwoodian	Gorstian
72	*Ruedemannia laevissima*	1	295	301	Laur	Homerian	Homerian
73	*Arjamannia aulongensis*	1	295	301	Laur	Homerian	Whitwellian
74	*Ruedemannia robusta*	7	282	330	Laur	Homerian	Gorstian
75	*Longstaffia tubulosa*	3	295	330	Laur	Homerian	Gorstian
76	*L. cyclonema*	5	302	308	Laur	Homerian	Homerian
77	*Trochonema fatua*	3	309	330	Laur	Gorstian	Gorstian
78	*Loxoplocus soluta*	4	309	330	Laur	Gorstian	Gorstian
79	*Lophospira cochleata*	3	309	330	Laur	Gorstian	Gorstian
80	*Trochonema muricata*	1	331	332	Laur	Ludfordian	Ludfordian
81	*Ruedemannia subrobusta*	3	331	337	Gond	Ludfordian	Ludfordian
82	*Ptychozone aberrans*	2	331	337	Gond	Ludfordian	Prídolí

APPENDIX 4

SYSTEMATIC PALEONTOLOGY

In the interest of conserving space, taxonomic revisions are presented only for new genera and for those requiring rediagnoses. Genera merely redefined but retaining traditional diagnoses are reclassified as per Fig. 14.

SUPERFAMILY LOPHOSPIROIDEA NOM. TRANS. WENZ, 1938

FAMILY LOPHOSPIRIDAE WENZ, 1938

Diagnosis: Lophospiroids primitively featuring a trilineate sinus keel, a strong left ramp carina, and a deep sinus that curves back to the sinus keel. All of these features change within the family in at least one subclade.

Included Genera: Lophospira, Donaldiella, Loxoplocus, Eunema, Gyronema, Ruedemannia, Arjamannia, Paupospira gen. nov., *Frodospira* gen. nov.

Genus *Eunema* Salter, 1859

Diagnosis: Prominent, sharp right and left ramp carina. Strong medial keel usually bordered by peripheral lira (although not in types species). Shallow sinus trending nearly straight into sinus keel. Increasing translation during final whorls, sometimes resulting in disjunct coiling.

Type Species: Eunema strigillata Salter, 1859.

Additional Species: "Lophospira" centralis, "L." helicteres, "L." concinula Ulrich and Scofield, 1897, *"L." quadrisulcata.*

Discussion. Knight *et al.* (1960) considered *Eunema* to be a polyphyletic subgenus of Trochonema, meant to describe high-spired variants of that genus. However, phylogenetic analyses indicate that the synapomorphies of *Eunema* and *Trochonema* are parallelisms. Knight *et al.* (1960) acknowledged the polyphyletic nature of the Trochonematidae by noting that the diagnostic characters (*e. g.* shallow sinus and a medial keel) appeared to be convergent among many forms. This analysis takes their conclusions a step further by positing that the form is polyphyletic among lophospiroids. The much more restrictive definition presented here excludes several species previously assigned to *Eunema*, which have been reassigned to genera such as *Globonema*.

First-known appearance (FKA): "Lophospira" centralis: Murfreesboro Limestone (Early Caradoc [Ashbyan]).

Last-known appearance (LKA): "Lophospira" quadrisulcata: Maquoketa Formation (Early Ashgill [Maysvillian]).

Genus *Loxoplocus* Fischer, 1885

Syn. *Kiviasukkaan* Peel, 1975b

Diagnosis: Sharp and strong medial keel that hooks slightly adapically, typically bordered by sharp but much weaker peripheral lira (although seemingly not on the type species). Narrow, shallow sinus, curving back towards sinus keel. Dull but strong left ramp carina, with right ramp concave and featuring a sharp carina at the suture, which creates an acute sutural margin. Increasing translation during final whorls, sometimes resulting in disjunct coiling (with the type species showing disjunct coiling throughout life).

Type Species: "Murchisonia" soluta Salter, 1859.

Additional Species: "Lophospira" sedgewicki Donald, 1906, *"L." ?bellicarinata* Donald, 1906, *"L." gotlandica* Ulrich and Scofield, 1897, *"Kiviasukkaan" nelsonae* Peel, 1975b.

Discussion: Knight *et al.* (1960) originally defined Loxoplocus as a broad genus that included *Lophospira* and other subgenera, an interpretation rejected by more recent authors (*e. g.* Tofel and Bretsky, 1987). It now is restricted to a paraclade of lophospiroids whose most prominent features include increasing translation over ontogeny, a very acute suture, and a very concave right ramp.

FKA: *"Lophospira" sedgewicki:* "Starfish Beds," Girvan District (Late Ashgill [Rawtheyan]).

LKA: *"Lophospira" gotlandica:* Kopanina Formation (Late Ludlow [Ludfordian]);

Genus *Paupospira* gen. *nov.*

Fig. 14

Diagnosis: Thick columella, filling the entire umbilicus and producing a shovel-like siphon in extreme cases. Deep and wide sinus curving back strongly to sinus keel. Sinus keel trilineate on early whorls, but with trilineations often vague and weak on adult whorls.

Type Species: Lophospira oweni Ulrich and Scofield, 1897.

Additional Species: "Lophospira" burginensis, "L." tenuistriata Ulrich and Scofield, 1897, *"L." sumnerensis* Ulrich and Scofield, 1897, *"L." tropidophora* Meek, 1873, *"Schizolopha" moorei, "Donaldiella" bowdeni* (Safford, 1869), *"Hormotoma" trilineata* Foerste, 1923.

Etymology: For David Swofford's computer program PAUP ("Phylogenetic Analysis Using Parsimony"; Swofford, 1998), which helped to diagnose and define this clade.

Discussion: Paupospira matches a monophyletic group originally hypothesized by Ulrich and Scofield (1897). A new genus is erected to recognize that group. The most distinguishing feature is the extremely thick columella, which often preserves as a core without the rest of the shell. Cladistic depictions of relationship highlighting the key features are given in Fig. 14.

FKA: *"Lophospira" burginensis:* Leray beds, Rockland Formation (Middle Caradoc [Rocklandian]).

LKA: *"Hormotoma" trilineata:* Saugh Hill Group (Middle Llandovery [Aeronian]).

Genus *Frodospira* gen. *nov.*

Diagnosis: Very small shells, with strong ornament that leaves imbrications on the growth lines, a strongly adapically curved medial keel, and highly tangential apertures.

Type Species: "Murchisonia" imbricata Lindström, 1884.

Additional Species: "Lophospira" munda (Lindström, 1884), *"L." cochleata* (Lindström, 1884).

Etymology: After J. R. R. Tolkein's character from *Lord of the Rings*, reflecting the unusually small size of the known species. Hypotheses about extraneous epidpodial tentacles are purely speculative.

Discussion: This closely matches Ulrich and Scofield (1897: 963) *imbricata* subsection of *Lophospira*. The genus is remarkable in that all known species are known only from very small shells. As these shells are found in the same assemblages as large gastropods, it appears to represent a character of the genus rather than a taphonomic artifact. The illustrations of these species, provided by Lindström (1884) are very accurate and convey the general characters very well.

FKA: *"Lophospira" munda:* C beds, Visby Formation (Early Wenlock [Sheinwoodian]).

LKA: *"Lophospira" imbricata:* Upper Hemse Beds (Early Ludlow [Gorstian]).

FAMILY TROCHONEMATIDAE ULRICH AND SCOFIELD, 1897

Diagnosis: Trochiform, widely umbilicate lophospiroids. Early forms retain wide, and deep sinus curving back to a trilineate selenizone, but later forms possess either the medial lira only or the peripheral lira only. Sharp left and right ramp carina with strong umbilical and sutural carina found on most species.

Included Genera: Trochonemella, Trochonema, Globonema.

Discussion: Knight *et al.*'s (1960) reduction of the Trochonematidae is advanced here. Preliminary analyses (Wagner, unpub. data) indicate that Devonian genera assigned to the Trochonematidae such as *Trochonemopsis* belong to the Euomphalinae. At this time, it is not clear if any post-Silurian genera assigned to this family belong here.

The Tonicella lineata (Wood, 1815) species complex (Polyplacophora: Tonicellidae), with descriptions of two new species

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Abstract: Four species of lined chitons from the Pacific coast of North America (two of them new) formerly regarded as *Tonicella lineata* (Wood, 1815) are described and discussed. *Tonicella lineata; T. undocaerulea* Sirenko, 1973; *T. lokii* Clark, *spec. nov.*; and *T. venusta* Clark, *spec. nov.* are differentiated by characteristics of their shells, girdle elements and radulae.

Key Words: *Tonicella lineata* complex, chitons, Polyplacophora

For many years chiton workers on the Pacific coast of North America have been aware of two or three varieties of lined chitons of the genus *Tonicella* Carpenter, 1873 that differed markedly from typical *T. lineata* (Wood, 1815). In 1973, Sirenko described *T. undocaerulea*, a species from the northern Sea of Japan that he believed to be the western Pacific Ocean counterpart of the eastern Pacific *T. lineata*. However, soon after its description, several workers recognized *T. undocaerulea* as one of the forms they had been encountering in the waters of Washington and British Columbia. In his revision of the family Lepidochitonidae of the eastern Pacific Ocean, Ferreira (1982) concluded that *T. undocaerulea* and the other lined forms were varieties of a single, widely distributed species properly referred to as *T. lineata*, a view echoed by Kaas and Van Belle (1985). My own preliminary investigations revealed many consistent differences between the various forms. These differences suggested the probability of a complex of several closely related species. Further investigation has produced evidence for the presence of four sibling species that overlap broadly in their ecological habitats as well as in their geographic and bathymetric ranges.

MATERIALS and METHODS

Several hundred specimens of *Tonicella* were examined and their characters and distributional patterns were compared. Valves, girdle elements and radulae were studied with light and scanning electron microscopes. Several specimens of each of four nominal species were immersed in a heated 10% solution of KOH until all that remained were the shell plates, radulae and epidermal layers (hypernotum & hyponotum) of the girdle; these were then rinsed thoroughly in distilled water. The shell plates were placed in a 50% solution of household bleach for 30 minutes, rinsed and air dried. Radulae and epidermal layers of the girdle were separately rinsed, dehydrated in an acetone series, and air dried. The specimens were then mounted on SEM stubs with colloidal silver paint, sputter coated with gold for two minutes, and examined at 5 or 10 KV with an Hitachi S-2100 scanning electron microscope at the Department of Biology at Southern Oregon University, Ashland, Oregon.

Abbreviations used in the text are: LACM, Los Angeles County Museum of Natural History; LACMIP, Los Angeles County Museum of Natural History-Invertebrate Paleontology; USNM, United States National Museum of Natural History, Washington, D.C.; CAS, California Academy of Sciences, San Francisco; SBMNH, Santa Barbara Museum of Natural History; ZISP, Zoological Institute, Saint Petersburg, Russia; RBCM, Royal British Columbia Museum, Victoria British Columbia, Canada; RMNH, Rijksmuseum van Natuurlijke Historie, Leiden; UMMZ, University of Michigan Museum of Zoology, Ann Arbor; UAF, University of Alaska, Fairbanks; RNC, Private collection of the author.

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SYSTEMATICS

Higher chiton systematics are presently in a state of flux. The widely used and variously modified scheme of Thiele (1909-1910) is based primarily on the characters of the shell plates. However, Sirenko (1993) has proposed a new scheme based on gill placement and the shape of egg hull processes. A very similar system of relationships had been noted earlier by Eernisse (1984). The new scheme appears to be an advancement and is herein adopted.

Order: CHITONIDA Thiele, 1929
Suborder: ACANTHOCHITONINA Bergenhayn, 1930
Superfamily: Tonicelloidea Simroth, 1894
Family: Tonicellidae Simroth, 1894
Subfamily: Tonicellinae Simroth, 1894
Genus: Tonicella Carpenter, 1873
Type species: *Chiton marmoreus Fabricius*, 1780, subsequent designation by Dall (1878).

Tonicella lineata (Wood, 1815)

(Figs. 1-8, 33)

Chiton lineatus Wood, 1815:15, pl. 2, figs. 4-5
Chiton (Stenosemus) lineatus Wood var. *fusca* Von Middendorff, 1847:110
Lepidochitona lineata: Oldroyd, 1927: 255, 256 (in part).
Tonicella lineata: Berry, 1917: 233 (in part); Sirenko, 1974: 995; Ferreira, 1982: 124, figs. 78-81 (in part); Baxter, 1983: 66; Kaas & Van Belle, 1985: 142-144, fig. 65, map 24 (in part); Baxter, 1987: 105.

Diagnosis: Chitons of medium size (to 5.0 cm), oval to elongate-oval, valves convex to subcarinate, beaked; tegmentum smooth (shiny) except for faint growth lines; color orange-salmon or maroon, with alternating white and dark maroon-brown lines; lines on head valve forming forwardly directed gothic arch or arrowhead shape.

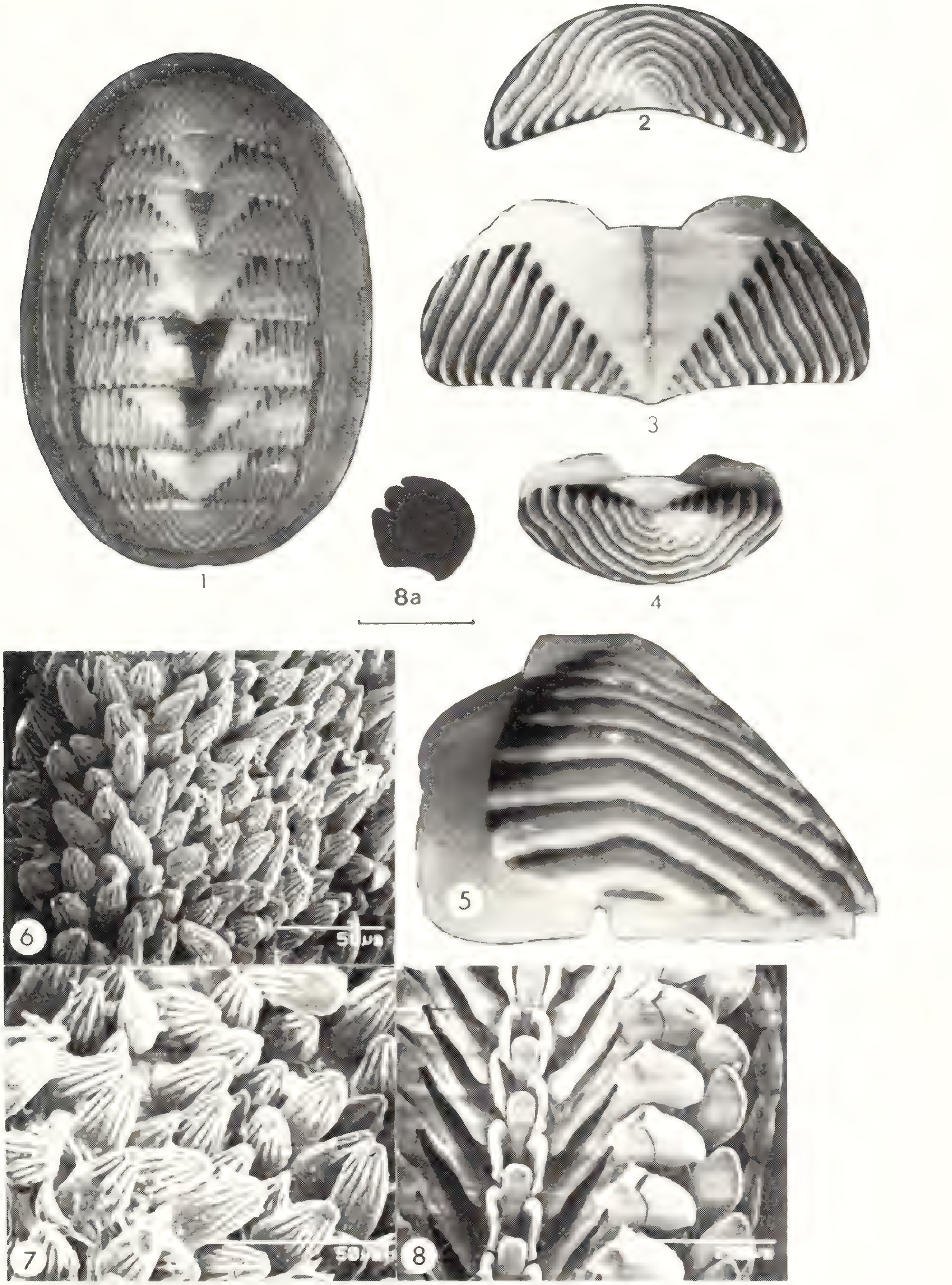
Description: Medium-sized chitons, often reaching 40-45 mm in length; largest specimen examined 52.5 mm (RNC 959, Ogden Point Breakwater, Victoria, British Columbia, Canada, *leg.* George P. Holm, 17 May, 1991). Shell (neotype, herein designated) (fig. 1) oval to elongate-oval, valves subcarinate to convex, beaked; tegmentum smooth (shiny), with faint growth lines. Head valve (fig. 2) semicircular, posterior margin widely V-shaped. Intermediate valves (fig. 3) broadly rectangular, lateral areas very slightly raised, side margins slightly rounded. Tail valve (figs. 4, 5) less than semicircular, about twice as wide as long, with mucro in anterior 1/5-1/3, post-mucronal slope straight to convex. Articulamentum white, with pink stain under central areas; sutural laminae short, wide, about 1/2 length of valve five tegmentum, moderately to strongly rounded; insertion teeth short, thick, finely rugose on anterior surface. Slit formula 8/1/9. Color: orange-salmon or maroon, with alternating white and dark, maroon-brown (often nearly black) lines on terminal valves and latero-pleural areas of intermediate valves; lines on head valve always forming forwardly directed arrowhead shape; jugal triangles delineated, usually of darker hues than ground color. Girdle of medium width, about 1/4 width of valve five tegmentum; usually light brown (in alcohol), often with paler bars but occasionally unicolored; dorsal surface covered with very minute, fairly broad, pointed scales (fig. 6), scales strongly ribbed on distal 1/2-3/4, about 28 μm long and 13 μm wide at base; ventral surface covered with similar but slightly larger scales (fig. 7) about 32 μm long and 15 μm wide. Radula (fig. 8) rachidian tooth narrow, elongate, sides nearly parallel along distal 3/4, distal end rounded; denticle cap of major lateral teeth (fig. 8a) broad, rounded, about 200 μm x 175 μm with deep notch on inner edge defining a small, thumb-like denticle, often with a smaller secondary notch above it. Ctenidia merobranchial, abanal, extending 4/5 length of foot.

Range of morphological vatiation: Some specimens have one or more dark maroon valves. Some specimens are nearly totally (except for subjugal markings) orange-salmon or maroon [var. *fusca* von Middendorff, 1847 (fig. 33), see discussion], but specimens nearly always have at least one white, dark maroon-brown, or black line on the head valve and often on the tail valve as well. Completely white "albino" specimens are infrequent.

Type locality: "Their country is unknown" (Wood, 1815: 16). Subsequently designated as Sitka, Alaska (Sirenko, 1974: 994). Here restricted to Old Sitka (Starrigaven Bay), 10 km N of Sitka, Baranof Id., Alexander Archipelago, SE Alaska (57°08'N, 135°55'W). Ferreira (1982: 125) designated Monterey Bay, California as the type locality, apparently unaware that Sirenko had already designated Sitka as the type locality.

Type material: Lost (Ferreira, 1982). Neotype LACM 2734 (fig. 1) here designated from type locality (leg. RNC, intertidal beneath cobble encrusted with coralline algae; 19 May, 1983); 35 mm x 22.5 mm x 6.5 mm (ex-RNC 496).

Additional material: Alaska: 1, CAS 41449, Montague Id.; 1, RNC 659, Navy breakwater, Kuluk Bay, Adak Id., Aleutian Islands, +0.5 m; 1, RNC 666, Nazan Bay, Atka Id., Aleutians, 0 m; 3, RNC 398, Chernofski



Figs. 1-8. *Tonicella lineata* (Wood, 1815). 1. Whole animal, Neotype, LACM 2734; Old Sitka, Baranof Island, Alaska. 35 mm x 22.5 mm x 6.5 mm. 2-8. RNC 430; Cape Arago, Oregon. 2. Head Valve; width 9.0 mm. 3. Intermediate valve five; width 12.2 mm. 4, 5. Tail valve; width 6.3 mm. 6. Dorsal girdle scales. 7. Ventral girdle scales. 8. Radula. 8a. Denticle Cap of Major Lateral Tooth. Bar = 200 µm.

Harbor, Unalaska Id., Aleutians, 0 m; 5, Eider Point, Unalaska Id., Aleutians, 0 m; 2, RNC 901, Herendeen Bay, SE Bering Sea, 0 m; 3, RNC 146, Chiniak Bay, Kodiak Id., 0 m; 4, RNC 442, Kachemak Bay, Kenai Peninsula, 0 m; 2, RNC 496, Old Sitka, Baranof Id., 0 m; 9, RNC 975, Petersburg, Mitkof Id., 0-1 m; 5, RNC 1295, Petersburg, Mitkof Id., 1 m; 6, CAS 083452, Edena Bay, Kosciusko Id.; 4, RNC 1236, N. Vallinar Rocks, Gravina Id., 3-5 m; 77, CAS 013454, N of Ketchikan, Revillagigedo Id.; 3, RNC 970, Saxman, Revillagigedo Id., 0-1 m; 3, RNC 969, Rotary Beach, Revillagigedo Id., 0-1 m; 3, RNC 1191, Mountain Pt., Revillagigedo Id., 0-1 m; 12, RNC 5450, Metlakatla, Annette Id., 0-2 m; 9, RNC 544, Brownson Bay, Prince of Wales Id., 0-1 m; 24, CAS 013121, Rose Inlet, Dall Id., British Columbia: 2, CAS 013590, Portage Inlet, 1 m; 5, RNC 934, Bowen Id., 3-5 m; 3, RNC 320, Victoria, Vancouver Id., 0-2 m; 1, RNC 959, Victoria, Vancouver Id., 0-1 m. Washington: 2, CAS 013346, Marrowstone Id., Jefferson Co.; 2, RNC 344, Indian Id., Jefferson Co., 0-1 m; 2, RNC 642, Neah Bay, Clallum Co., 0-1 m; 5 RNC 13. Tacoma Narrows, Pierce Co., 0-1 m. Oregon: 2, RNC 430, N Cove, Cape Arago, Coos Co., +1 m; 5, RNC 4, Cape Blanco, Curry CO., 0 m; 1, RNC 979, Port Orford, Curry Co., 2 m; 1, RNC 999, Island Rock, Curry Co., 17 m; 4, RNC 963, Brookings, Curry Co., 0 m. California: 4, CAS 060413, Trinidad, Humboldt Co,; 28, CAS 009294, S of Cape Mendocino, Humboldt Co.; 3, CAS 013396, Shelter Cove, Mendocino, Co., 0-1 m; 1, CAS 013374, Fort Ross, Sonoma Co.; 2, CAS 077112, Bodega Head, Sonoma Co,; 3, CAS 069442, Bodega Bay breakwater, Sonoma Co.; 4, CAS 034724, Duxbury Reef, Marin Co.; 2, CAS 078781, Marin Co.; 1, CAS 077028, Angel Id., San Francisco Bay; 3, CAS 001933, Fort Mason , San Francisco Co.; 2, CAS 007149, Franklin Point, San Mateo Co.; 1, CAS 007148, Ano Nuevo Point, San Mateo Co.; 1, CAS 000219, Davenport Landing, Santa Cruz Co.; 3, CAS 013361, China Point, Monterey Co.; 2, RNC 1233, Coast Guard breakwater, Monterey Bay, 3-5 m.

Distribution: Tonicella lineata is a North American boreal species endemic to the Aleutian and Oregonian provinces (Fig. 37). The species occurs continuously from the central Aleutian Islands to central Californica in depths ranging from +2 to 17 m. The westernmost record is Navy breakwater, Kuluk Bay, Adak Island, Aleutian Islands, Alaska (51°45'N, 176°45'W) (LACM 86-00.0, RNC 659, leg. Rae Baxter, 12 August, 1286, intertidal); the northernmost record is Montague Island, Prince William Sound, Alaska (60°10'N, 147°15'W) (CAS 41449), and the southernmost record is the United States Coast Guard breakwater, Monterey Bay, Monterey County, California (36°45'N, 121°55'W)(RNC 1060; leg. RNC, with SCUBA, 23 March, 1992, 5 m). These animals are abundant at some localities, with population densities often exceeding 50 per square m. This species fairly common at the Navy breakwater at Adak Island (Rae Baxter, pers. comm., Nov. 1988) but is abruptly absent west of that locality. In the southern portion of its range, it becomes uncommon from Mendocino County, California southward, where it begins to overlap with the similar *Tonicella lokii*; it is quite rare in Monterey County.

Habitat and ecology: Lives on cobbles, boulders and bedrock encrusted with purple-pink coralline red algae (*Lithothamnion* spp.) or unidentified rust-brown encrusting bryozoans

Fossil record: Valves of *Tonicella lineata* have been identified from Pleistocene deposits in southern Oregon and northern California (LACMIP loc. 2636, Coquille Point, Coos Co., Oregon; LACMIP loc. 2641, Cape Blanco, Curry Co., Oregon; LACMIP loc. 3955, Battery Formation, Crescent City, Del Norte Co., California; LACMIP loc. 4816 & 10770 & USGS loc. M7824, Point Arena, Mendocino Co., California). These specimens have been radiometricaly dated at 80,000-85,000 years before present (BP). Specimens from Moonstone Beach, Humboldt Co., California (LACMIP loc. 3942) have been dated at 700,000-1,000,000 years BP (George L. Kennedy, pers. comm. Oct. 1992).

Remarks: *Tonicella lineata* is remarkably similar to *T. undocaerulea* Sirenko, 1973 and *T. lokii* Clark, spec. nov. (see comments for those species).

Tonicella undocaerulea Sirenko, 1973

(Figs. 9-16, 35, 36)

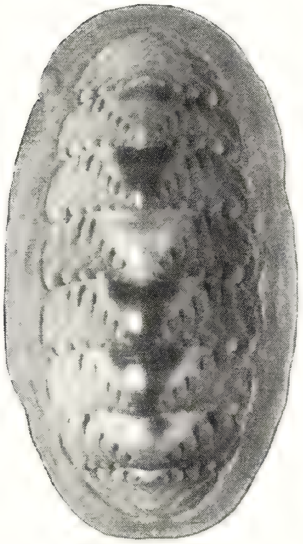
Lepidochitona lineata, non Wood: Oldroyd, 1927: 255, 256 (in part).

Tonicella lineata, non Wood: Taki, 1938: 331; Yakovleva, 1952: 56, 61-62 (in part); Itoigawa *et al.*, 1978 (Fossil): 150-153, pl. 16, figs. 2-7; Ferreira, 1982: 124-126 (in part); Kaas & Van Belle, 1985: 142-144, fig. 65-5, 6, map 24 (in part).

Tonicella undocaerulea Sirenko, 1973: 663, figs. 3/1-7, 9-13; Baxter, 1983: 66; Baxter, 1987: 105.

Diagnosis: Chitons of relatively small size [(to 3.8 cm), Asian specimens smaller]; shell oval to elongate-oval; valves subcarinate to round, beaked; tegmentum smooth, shiny; color (in alcohol) light orange with white zigzag lines, often with short, dark maroon streaks on edges of pleural areas.

Description: Relatively small chitons, rarely exceeding 25 mm in North American waters and 16 mm in



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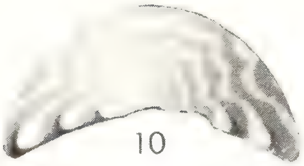

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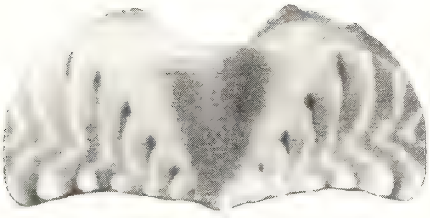

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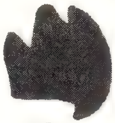

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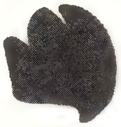

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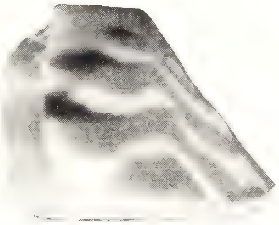

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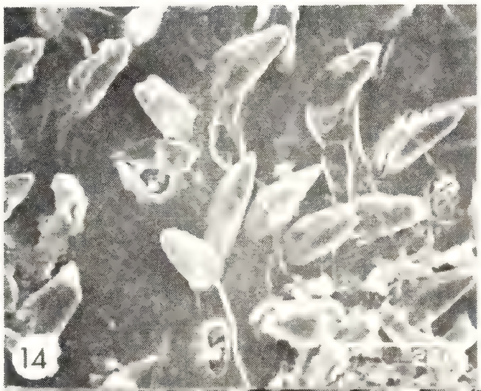
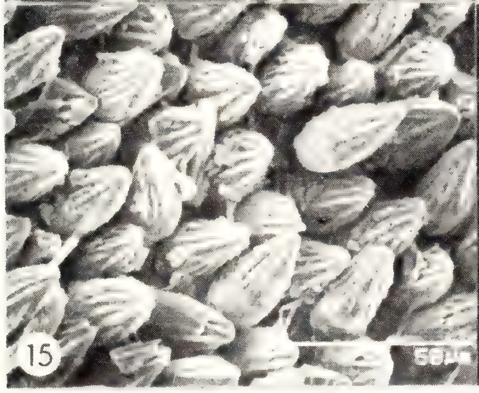
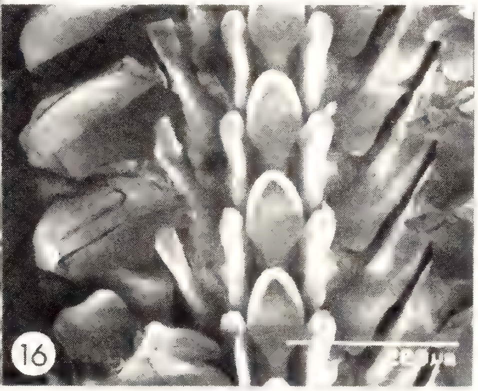






Figs. 9-16. *Tonicella undocaerulea* Sirenko, 1973. 9. Whole animal, RNC 2222; Vallinar Rocks, Gravina Id. (near Ketchikan) Alaska. 25.0 mm x 14.0 mm x 4.5 mm. 10-16 RNC 255; Neah Bay, Clallum County, Washington. 10. Head valve; width 4.5 mm. 11. Intermediate valve five; width 6.7 mm. 12, 13. Tail valve; width 4.0 mm. 14. Dorsal girdle scales. 15. Ventral girdle scales. 16. Radula. 16a. Denticle Cap of Major Lateral Tooth (Asian specimen). 16b. Denticle Cap of Major Lateral Tooth (North American specimen). Bar = 100 µm.

Asian waters. Largest specimen examined 38.0 mm (RNC 287b, Ogden Point breakwater, Victoria, British Columbia, *leg*. RNC, 12 July, 1987). Shell oval to elongate-oval (often egg-shaped)(fig. 9), valves subcarinate to round, beaked; tegmentum smooth, shiny, usually lacking noticeable growth lines. Head valve (fig. 10) semicircular, posterior margin widely V-shaped to nearly straight. Intermediate valves (fig. 11) broadly rectangular, lateral areas indistinct to slightly raised, side margins slightly rounded. Tail valve (figs. 12, 13) oval, mucro in anterior 1/3, postmucronal slope convex to (rarely) slightly concave. Articulamentum white, stained with intense rose-maroon under central areas; sutural laminae short, wide, about 1/3 length of valve five tegmentum, rounded; insertion teeth short, solid; slit formula 8/1/8-9. Color light orange, terminal valves with concentric, white zigzag lines radiating from apices; lateropleural areas with white zigzag lines, those on pleural areas often bordered beneath with short, dark maroon streak; jugal areas with delineated triangles of maroon, yellow, orange, or white. Girdle narrow, less than 1/4 width of valve five tegmentum, appearing nude; dorsal surface covered with minute, rather narrow, pointed, smooth scales (fig. 14), about 18-20 µm long and 5 µm wide, with single groove (often obscured) extending from apex to about mid length of scale; ventral surface covered with minute, tear-drop shaped scales (fig. 15), about 25 µm long and 12 µm wide, ribbed along proximal 1/2-1/3. Radula (fig. 16) rachidian tooth very broadly dilated anteriorly, forming distinctive spoon shape; denticle cap of major lateral teeth (16a & b) tridentate, about 120 µm x 130 µm, central cusp the largest, inner cusp slightly smaller, and outer cusp about 1/2 length of the central one. Ctenidia merobranchial, abanal, extending about 3/4 to 4/5 length of foot.

Range of morphological variation: *Tonicella undocaerulea* varies somewhat in shape and size from one locality to another in American waters, some specimens being quite egg-shaped in outline, whereas others are very elongate. The slope of the shell also varies from straight and nearly carinate to very convex. The valves of some specimens may be completely maroon, or suffused with maroon on the lateropleural areas; the ground color of some specimens may be yellow.

Type Locality: Russia, Bay of Minonosok, Posjet Strait, Sea of Japan (42°39'N, 130°54'E), by original designation (Sirenko, 1973).

Type material: Holotype ZISP 1905; paratypes ZISP 1906 & 1907.

Additional material: Russia: 3, RNC 103, Vostok Bay, Sea of Japan, 1-2 m; 3, RNC 118, Vostok Bay, Sea of Japan, 1 m; 2, CAS 077081, Vostok Bay, Sea of Japan, 1-22 m; 7, CAS 013448, Vostok Bay, Sea of Japan, 2-3 m; 12, LACM 91-91, Vostok Bay, Sea of Japan, 0-3 m; 4, LACM 91-94, Vostok Bay, Sea of Japan, 3-10 m; 1, LACM 91-89, Popova Id., Amuskly Bay, Sea of Japan, 0-2 m. Japan: 8, LACM 82-12, Mutsu Bay, Honshu Id., 3-10 m; 1, RNC 725, Oshoro, Hokkaido, 1-3 m; 1 valve, RNC F-1 (fossil), Soli, Kisarazu City, Chiba Prefecture, Kioroshi Formation, Shimosa group, Middle Pleistocene. Alaska: 2, RNC 501, Naked Id., Prince William Sound, 3-6 m; 1, CAS 013449, Kodiak Id.; 1, RNC 1196, Chiniak Bay, Kodiak Id., 1 m; 1, RNC 679, Hesketh Id., Kachemak Bay, Kenai Peninsula, 9 m; 6, RNC 348, Kachemak Bay, Kenai Peninsula, 1-2 m; 6, RNC 348, Kachemak Bay, Kenai Peninsula, 1-2 m; 6, RNC 144, Sitka, Baranof Id., 1-2 m; 1, LACM 73-13, Sitka, Baranof Id., 1-5 m; 1, LACM 73-15, Pirate Cove, Baranof Id., 3-12 m; 2, RNC 1192, Petersburg, Mitkof Id., 5-6 m; 25 CAS 013331, Edena Bay, Kosciusko Id.; 5 RNC 2222, Vallinar Rks., 3-5 m; 3 RNC 940, Saxman, Revillagigedo Id., 0-2 m; 5, RNC 1031, Rotary Beach, Revillagigedo Id., 0-1 m; 4, RNC 241, Mountain Pt., Revillagigedo Id., 1-3 m; 10, RNC 1189, Mountain Pt., Revillagigedo Id., 0-5 m; 9, RNC 425, Metlakatla, Annette ID., 0-2 m; 1, RNC 1198, Washington Monument, Revillagigedo Channel, 5-8 m. British Columbia: 1, RNC 222, Port Hardy, Vancouver Id., 1-2 m; 1, CAS 002414, Quiet Bay, Vancouver Id.; 11, RNC 287, Victoria, Vancouver Id., 0-2 m. Washington: 5, RNC 136, San Juan Id., 12-18 m; 2, RNC 337, Port Gable, Hood Canal, Kitsap Co., 1 m; 2, RNC 255, Neah Bay, Clallum Co., 1-2 m. Oregon: 5, RNC 973, Island Rock, Curry Co., 30 m; 1, RNC 141, Brookings, Curry Co. California: 9, LACM 71-106, Tolo Bank, Mendocino Co., 21-30 m; 1, LACM 64-20, Salt Point Ranch, Sonoma Co., 3-5 m; 1, CAS 008948, SE Farallon Id.; 4, CAS 013350, Monterey Bay, Monterey Co., 18 m; 9, LACM 60-22, Del Monte, Monterey Bay, 18 m; 6, RNC 1027, Coast Guard breakwater, Monterey Bay, 3-15 m; 3, LACM 63-3, Coast Guard breakwater, Monterey Bay, 4-12 m; 2, CAS 083441, Chace Reef, Monterey Co., 12-13 m; 1, LACM 60-24, Carmel Submarine Canyon, Monterey Bay, 3-15 m; 3, LACM 63-3, Coast Guard breakwater, Monterey Bay, 4-12 m; 2, CAS 083441, Chace Reef, Monterey Co., 12-13 m; 1, LACM 60-24, Carmel Submarine Canyon, Monterey Bay, 12-38 m; 2, LACM 38-153, San Luis Obispo Bay, San Luis Obispo Co., 15-26 m; 1, LACM 38-162, San Miguel Id., Channel Islands, 9-27 m.

Distribution: *Tonicella undocaerulea* has a discontinuous distribution. In Asian waters it has been reported from the Sea of Japan (Sirenko, 1973; Saito, 1994) from Mutsu Bay, Honshu Id. Japan (42°20'N, 140°55'E) to near Uglegorsk, SE Sakhalin Id., Russia (49°00'N, 142°31'E) and the Okhotsk Sea (Sirenko, 1973) from Iturup Id., Kurile Is., Russia (approx. 45°00'N, 147°30'E) at depths of 1-27 m (Sirenko, 1973). In American waters it is found from Kodiak Id., Alaska (57°50'N, 152°30'W)(RNC 1196) and Naked Id., Prince William Sound, Alaska (60°37.8'N, 146°23'W)(RNC 501) to San Miguel Id., California (34°01'N, 120°24'N)(LACM 38-162) (Fig. 37) at depths of 0-38 m.

Habitat: *Tonicella undocaerulea* lives on encrusting coralline algae (*Lithothammon* spp.) on pebbles, cobbles, boulders and bedrock.

Fossil record: Valves of *T. undocaerulea* have been found in the middle Pleistocene deposits on the Boso Peninsula, Honshu Id., Japan (Itoigawa *et al.*, as *T. lineata*).

Remarks: *Tonicella undocaerulea* has long been confused with the similar *T. lineata* and *T. lokii* because of its lined color pattern and habitat of encrusting *Lithothamnion*. However, it may be distinguished from both of theses species by its lack of maroon-brown "black" lines on the valves.

North American specimens of *Tonicella undocaerulea* are considerably larger than Asian specimens, attaining a length of up to nearly 40 mm, whereas the largest specimen recorded from Asia is 16 mm (Sirenko, 1973). Also, the denticle cap of the major lateral teeth of North American specimens have very rounded cusps, in Asian specimens they are quite triangular. However, all other characters of North American and Asian specimens are identical. Because of the differences in size and distribution, and the shape of the denticle caps, North American specimens should perhaps be considered a subspecies.

Tonicella lokii sp. nov.

(Figs. 17-24)

Tonicella lineata, *non* Wood: Berry, 1922: 433, pl. 2 figs. 1-5 (fossil); Burghardt and Burghardt, 1969: 36 (in part, pl. 4, no. 76); of authors (from Monterey Bay and central California).

Lepidochitona lineata, non Wood: Oldroyd, 1927: 255, 256 (in part).

Diagnosis: Chitons of medium size (to 5.0 cm), shell low to moderately elevated; valves salmon to light orange, with white and dark maroon-brown (black), zigzag lines on terminal valves and lateropleural areas of intermediate valves; jugal areas with dark orange, pink, or maroon triangles; tail valve with concave post-mucronal slope; girdle with alternating light and dark bars.

Description: Holotype (fig. 17) 32.3 mm x 21.0 mm x 5.5 mm; body oval in outline, slightly elevated; valves subcarinate, smooth. Head valve (fig. 18) semicircular, posterior margin widely V-shaped. Intermediate valves (fig. 19) rectangular, length about 1/3 width, slightly beaked. Tail valve (figs. 20-21) oval, more than twice as wide as long, mucro in anterior 1/3, post-mucronal slopes concave. Articulamentum white, with light brown rays on slit rays, often with triangular pink stain under central areas; slit formula 9/1/11; sutural laminae short, about 1/2 length of valve five tegmentum; jugal sinus rather narrow; insertion teeth short, thick, those on head valve crenulated on exterior surface. Girdle moderately wide, about 1/4 width of valve five tegmentum; dorsal surface appearing smooth, actually clothed with very minute non-imbricating, broad, smooth, bluntly pointed scales (fig. 22) 22-25 µm long and 10-12 µm wide, rarely with a barely perceptible groove extending from apex to mid-length of scale; ventral surface covered with minute, densely packed, broad scales (fig. 23) 2/3 length. Radula (fig. 24) 9.7 mm long, bearing 53 mature rows of teeth; rachidian tooth about 100 µm long, narrow at base dilated anteriorly to broad, spatula shape about 50 µm wide and slightly recurved at distal end; denticle cap of major lateral teeth (24a) broad, rounded, slightly longer than wide, about 120 µm x 135 µm, with deep notch on inner edge defining a small thumb-like denticle. Ctenidia merobranchial, abanal, extending 4/5 length of foot, about 25 plumes per side.

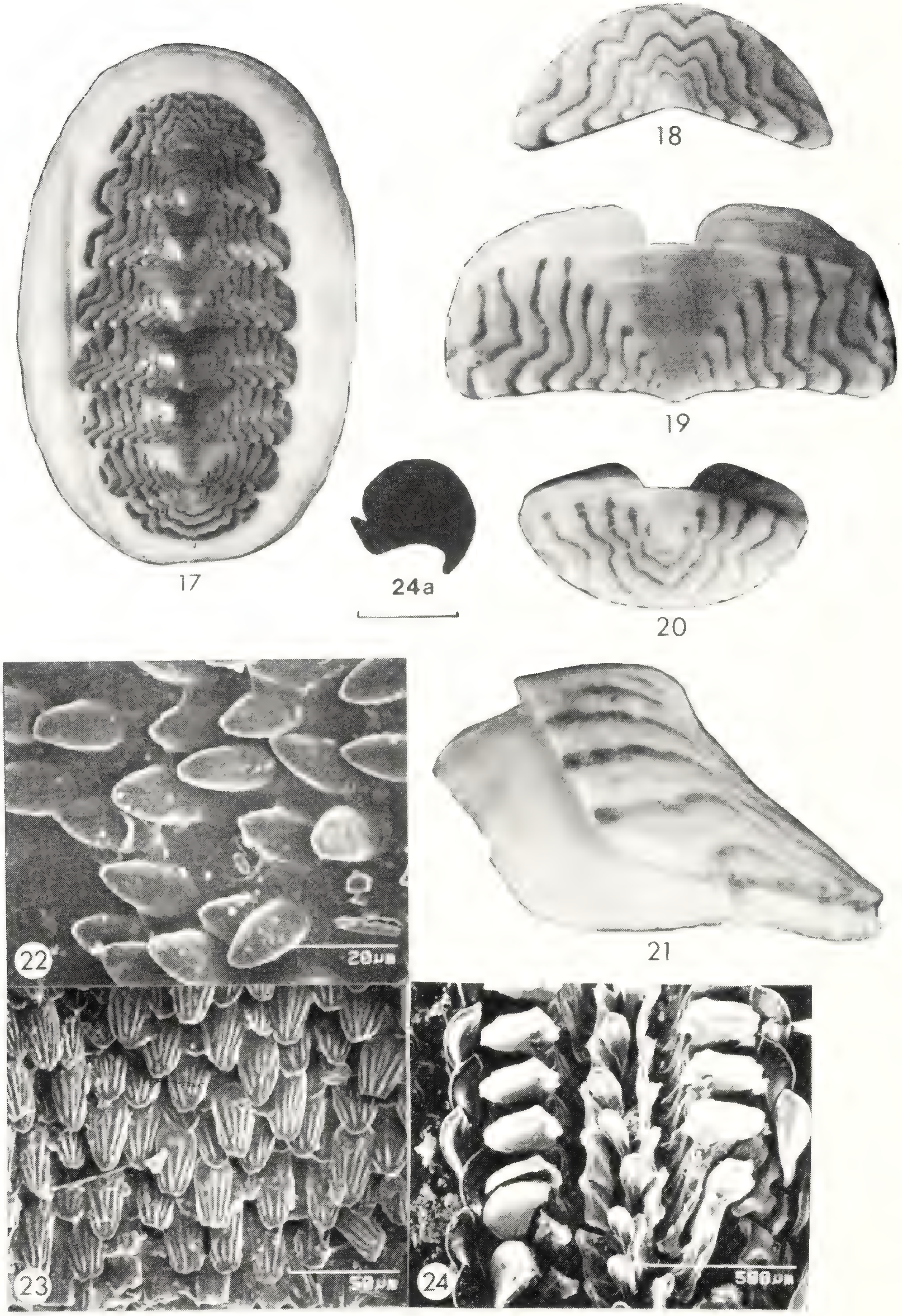
Range of morphological variation: As is the case with several members of this genus, individual valves may be uniformly white or dark maroon. However, unlike other *Tonicella* spp., the central portion of the articulamentum is often unstained, and when stain is present it may be pink, rose, or light brown.

Type locality: Coast Guard breakwater, Monterey Bay, Monterey County, California (36°45'N, 121°55'W), 0-13 m.

Type material: Holotype (LACM 2623) and 36 paratypes (2, LACM 2624 & 2625); (2, USNM 880067); (2, CAS 103560); (2, SBMNH 141109); (1, UMMZ 252868); (2, RMNH 9360); (3, ZISP 1933); (22, RNC 1056 & 1191).

Type material preserved flat and fully extended, all but seven in 70% ethanol or 70% isopropyl alcohol; remaining seven specimens glycerin-dried. Type material collected 16 & 23 March, 1992 by RNC (9 specimens) and 26 March, 1993 by RNC and Bob Abela (28 specimens).

Additional material: California: 1, CAS 013583, Shelter Cove, Humboldt Co.; 1, CAS 013798, N of West-


18
17
19
24a
20
21
22
20µm
23
50µm
24
500µm

Figs. 17-24. *Tonicella lokii* Clark, spec. nov. 17. Whole animal, Holotype, LACM 2623; 32.3 mm x 21.0 mm x 5.5 mm. 18-24. Paratype, RNC 1056. 18. Head valve; width 9.4 mm. 19. Intermediate valve five; width 13.2 mm. 20, 21. Tail valve; width 8.8 mm. 22. Dorsal girdle scales. 23. Ventral girdle scales. 24. Radula. 24a. Denticle Cap of Major Lateral Tooth. Bar = 100 µm.

port Landing, Mendocino Co.; 3, CAS 013459, Buckhorn Cove, Mendocino Co., 0.5 m; 5, CAS 013453, Albion Head, Mendocino Co.; 10, LACM 49-3, Salmon Point, Mendocino Co., 0.5 m; 20, LACM 50-11, Fort Ross, Sonoma Co., 0.5 m; 8, CAS 043848, Stewart's Point, Sonoma Co.; 3, CAS 069441, Bodega Bay breakwater, Sonoma Co.; 1, CAS 043485, Tomales Point, Marin Co.; 18, CAS 008948, SE Farallon Id.; 1, CAS 069445, S of Pescadero Point, San Mateo Co.; 1, CAS 007065, Pigeon Point, San Mateo Co.; 1, CAS 000217, Davenport Landing, Santa Cruz Co.; 5, CAS 013350, Monterey Bay, Monterey Co., 18 m; 6, CAS 013361, China Point, Monterey Co.; 2, CAS 013364, Cannery Row, Monterey Bay, 10 m; 7, LACM 63-58, Mission Point, Monterey Co., 0.5-2 m; 2, RNC 342, Point Pinos, Monterey Co., 0-1 m; 4, RNC 656, Carmel, Monterey Co., 0-1 m; 2, CAS 056335, 6.4 km N of San Simeon, San Luis Obispo Co.; 2, RNC 402, Cayucos, San Luis Obispo Co., 0.5 m; 1 CAS 013586, Purisima Point, Santa Barbara Co.; 1, CAS 020482, Surf, Santa Barbara Co.; 1, LACM 64-28, S side of Anacapa Id., Channel Is., 15-24 m; 7, LACM 67-38, SE of Bay Point, San Miguel Id., Channel Is., 0-8 m.

Distribution: *Tonicella lokii* occurs between latitudes 40°N and 34°N , on the northern and central coast of California (Fig. 37) at depths of 0-24 m. The northernmost record is Shelter Cove, Humboldt County (40°01.5'N, 124°45'W)(CAS 013583), and the southernmost record is SE of Bay Point, San Miguel Id., California (34°02'N, 120°18'W)(LACM 67-38). Only one specimen of T. lokii has been recorded from Humboldt County, and the species is not common in adjacent Mendocino County to the south, but it becomes more abundant closer to the Monterey County distribution center.

Habitat and ecology: *Tonicella lokii* lives on cobbles, boulder, and bedrock encrusted with coralline algae (*Lithothamnion* spp.).

Fossil record: Valves of *T. lokii* have been identified from Pleistocene deposits in southern California (LACMIP Loc. 11004, First Terrace, Army Camp Beach, San Nicholas Island), at a San Pedro area terrace (Berry, 1922, as *T. lineata*), and from northern Baja California, Mexico (LACMIP loc. 10131, Lighthouse Terrace, Bahia el Playon, Punta Banda, and LACMIP loc. 10619, Lighthouse Terrace, near tip of Punta Banda). The specimens have been dated at 80,000-85,000 years BP (G. L. Kennedy, pers. comm., 1992).

Etmology: Named for Loki, the Norse God of mischief and deception, appropriate for a species that long has deceived biologists as to its true identity.

Remarks: *Tonicella lokii* is often found with the morphologically similar and apparently closely related *T. undocaerulea*, but it can be easily distinguished from the latter by the presence of dark maroon-brown lines on its valves. *Tonicella lokii* may also be found with *T. lineata*, from which it can be distinguished by the concentric zigzag lines on the head valve (those on *T. lineata* form a very distinctive gothic arch) and by the concave post-mucronal slope of its tail valve.

Tonicella venusta sp. nov.

(Figs. 25-32, 34)

Tonicella rubra, *non* Linnaeus: Berry, 1917: 233 (in part); Smith and Gordon, 1948: 205; Burghardt and Burghardt, 1969: pl. 4, fig. 79.

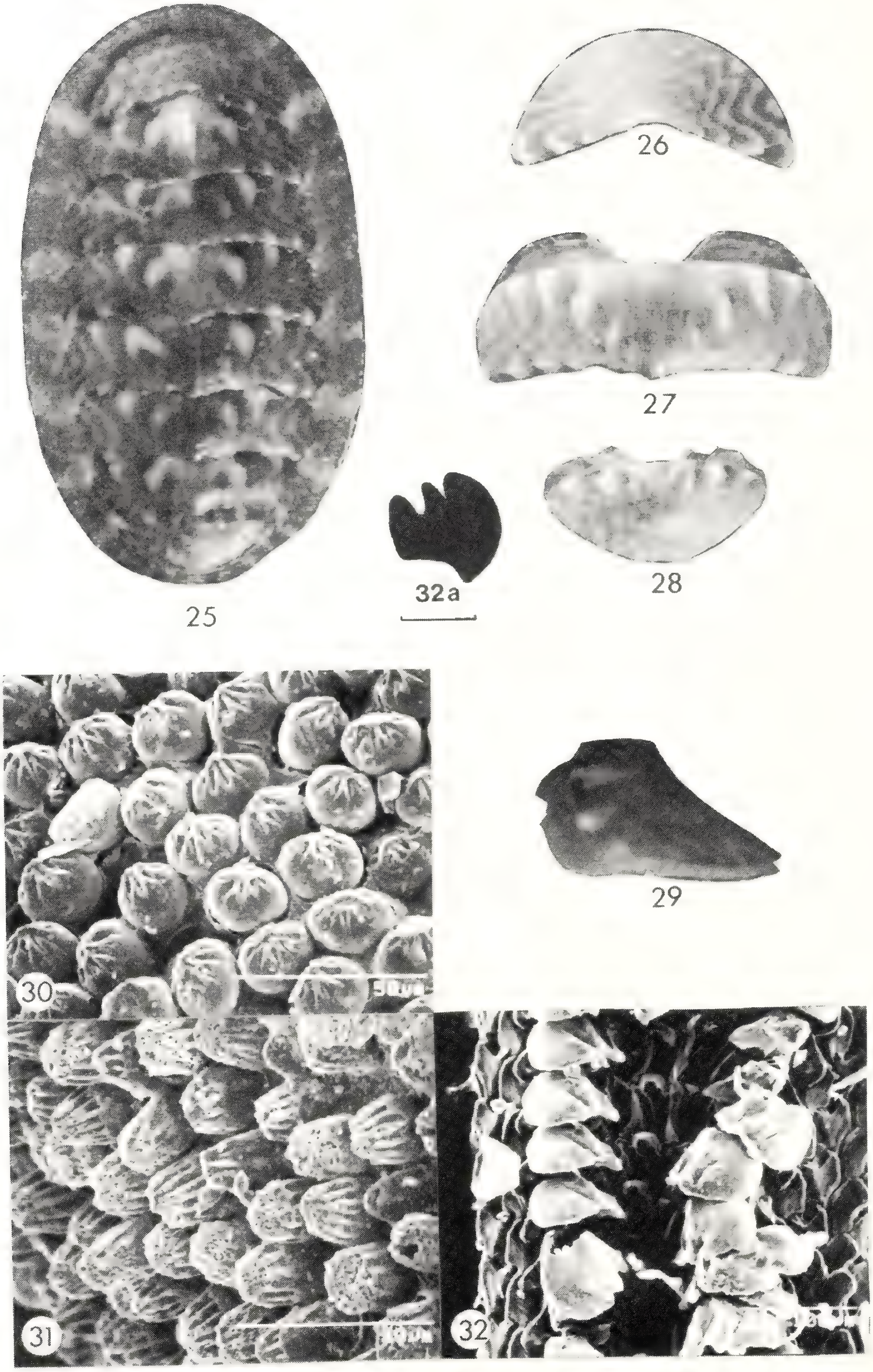
Lepidochitona lineata: Oldroyd, 1927: 255, 256 (in part).

Lepidochitona ruber, non Linnaeus: Oldroyd, 1927: 256-257 (in part).

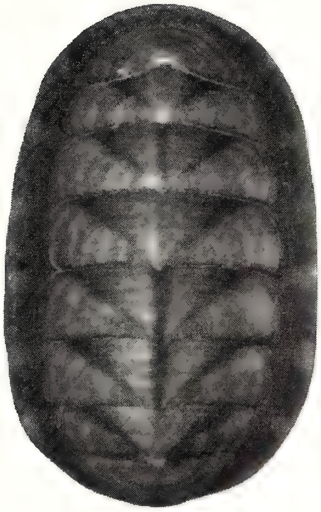
Tonicella marmorea, *non* Fabricius: Baxter, 1983: 66.

Diagnosis: Chitons of small size (to 1.7 cm), oval, shell low to moderately elevated, subcarinate; valves light orange or pink, terminal valves and lateral areas of intermediate valves with white, zigzag lines; pleural areas with 2-5 large white flammules; jugal areas with orange, pink, white or maroon triangles; post-mucronal slope of tail valve concave.

Description: Holotype (fig. 25) preserved dry, flat and fully extended, 10.5 mm x 6.0 mm x 1.8 mm; body oval in outline, slightly elevated; valves subcarinate, smooth. Head valve (fig. 26) semicircular, posterior margin widely V-shaped. Intermediate valves (fig. 27) rectangular, length about 1/3 width, beaked; lateral areas poorly defined. Tail valve (figs. 28-29) oval, length about 1/2 with; mucro anterior 1/3, post-mucronal slope concave. Articulamentum white or pale pink, with triangular maroon stain under central area; sutural laminae short, about 1/3 length of valve five tegmentum; jugal sinus about 1/5 valve width; insertion teeth short, fairly thick; slit formula 8/1/11. Girdle of moderate width, about 1/4 width of valve five tegmentum, of sandy appearance, clothed dorsally with relatively large, closely packed, erect, smooth, rotund, mammilate scales (fig. 30) about 45 µm long and 28 µm wide; mammilae with 8-10 heavy ribs; ventral surface of girdle covered with closely packed, rather broad, bluntly tapering scales (fig. 31) about 30 µm long and 16 µm wide, strongly ribbed on proximal 1/3-1/2. Radula (fig. 32) 3.2 mm long, with 53 mature rows of teeth; rachidian tooth spatulate, about 75 µm long and 12 µm wide at base, dilating proximally to about 30 µm wide at working edge, thickened on



Figs. 25-32. *Tonicella venusta* Clark, spec. nov. 25. Whole animal, Holotype, LACM 2626: 10.5 mm x 6.0 mm x 1.8 mm. 26-32. Paratype, RNC 1033 26. Head valve; width 4.0 mm. 27. Intermediate valve five; width 5.0 mm. 28, 29. Tail valve; width 3.2 mm. 30. Dorsal girdle scales. 31. Ventral girdle scales. 32. Radula. 32a. Denticle cap of major lateral tooth. Bar = 50 µm.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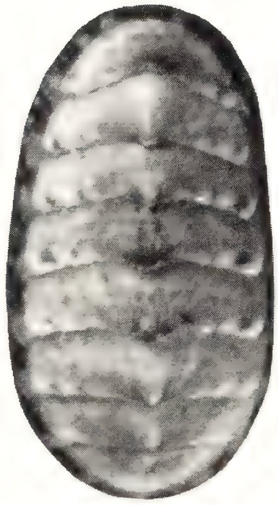
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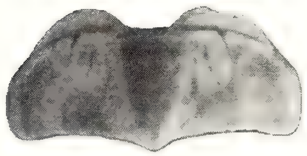
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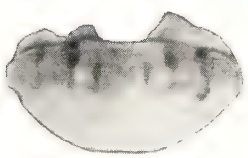
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Fig. 33. *Tonicella lineata* var. *fusca* (von Middendorff, 1847) ? RNC 934, Tunstal Bay, Bowen Id., near Vancouver, British Columbia, 6 m. 38.0 mm x 23.0 mm x 6.0 mm. **Fig. 34.** *Tonicella venusta* Clark, spec. nov., RNC 194, Neah Bay, Clallum Bay, Washington, 0-1 m. 8.0 mm x 4.0 mm x 2.0 mm (Albino). **Figs. 35 & 36.** *Tonicella undocaerulea* Sirenko, 1973; fossil intermediate and tail valves, middle Pleistocene, Boso Peninsula, Honshu Id., Japan (Photos: courtesy of A. Naruse). Scale bar 5.0 mm.

lateral edges and notched at center at proximal end; denticle cap of major lateral teeth (32a) broad, tridentate, about 65 µm x 75 µm, rounded on outer edge, denticles about equal in length (inner one only slightly smaller). Ctenidia merobranchial, abanal, extending about 3/4 length of foot, 16 plumes per side.

Range of morphological variation: Some specimens have a dark maroon-brown, central area bordered by a diagonal white band at the edge of the lateral areas on valve two. Rare albinistic specimens (fig. 34) are gray-white with a few darker gray blotches at the posterior edges of the plates. Some specimens are nearly uniformly, light pink, and one southern specimen has the color pattern of the lateropleural areas extending across the jugal areas.

Type locality: Mountain Point, 8 km S of Ketchikan, Revillagigedo Island, Alexander Archipelago, SE Alaska (55°17’35”N, 131°32’20”W), 1-10 m.

Type Material: Holotype (LACM 2626) and 41 paratypes (2, LACM 2627, 2628); (2, CAS 103559); (1, SBMNH 141110); (2, USNM 880068); (1, RMNH 9361); (1, ZISP 1934); (2, UAF MO-5572); (1, UMMZ 252870); (29, RNC 1026, 1033, 1140).

Type material preserved flat and fully extended. Holotype and 17 paratypes preserved dry (with glycerin), collected 24-25 June, 1991 by RNC & Alan Murray; Twenty-four additional paratypes preserved in 70% ethanol, 8 collected 3 September, 1992 by RNC, Alan Murray, Kurt Morin & David Zwick, and 16 collected 26-30 September, 1993 by RNC, David Zwick & Kurt Morin.

Additional Material: Alaska: 1, CAS 025595, Knight Id., Prince William Sound; 5, LACM 83-106, Hesketh Id., Kachemak Bay, Kenai Peninsula, 12-16 m; 3, RNC 435, Kachemak Bay, 0-2 m; 2, RNC 1193, Chiniak Bay, Kodiak Id., 1 m; 7, RNC 200, Sitka, Baranof Id., 0-1 m; 1, RNC 1193, Chiniak Bay, Kodiak Id., 1 m; 7, RNC

200, Sitka, Baranof Id., 0-1 m; 1, RNC 1197, Petersburg, Mitkof Id., 8-10 m; 2, SBMNH 36067, Forrester Id.; 6, RNC 1010, Rotary Beach, Revillagigedo Id., 0-1 m; 9, RNC 1033, Mountain Pt., Revillagigedo Id., 1 m; 9 RNC 579, Metlakatla, Annette Id., 42 m; 1, RNC 912, Saxman, Revillagigedo Id., 1 m; 9, RNC 1199, Washington Monument (submerged pinnacle), Revillagigedo Channel, 5-30 m. British Columbia: 1, SBMNH 36069, Departure Bay, Vancouver Id.; 3, RNC 251, Tofino Harbor, Vancouver Id., 1-2 m; 8, RBCM 7516, Tofino Harbor, Vancouver Id., 1-2 m; 2, RNC 397 Saanach Inlet, Vancouver Id., 18 m; 2, RNC 349, Victoria, Vancouver Id., 1-2 m. Washington: 6, RNC 194, Neah Bay, Clallum Co., 0-1 m. Oregon: 1, RNC 972, Port Orford breakwater, Curry Co., 5 m; 1 RNC 971, Island Rock, Curry Co., 30 m. California: 1, CAS 013476, Shelter Cove, Mendocino Co.; 1, RNC 1226, Coast guard breakwater, Monterey Bay, Monterey Co., 10-12 m; 3 SBMNH 36073, N of San Simeon, San Luis Obispo Co.; 1, Hanselman Coll., Gaviota, Santa Barbara Co., 18 m; 1, LACM 41-195, E of Carwell Point, San Miguel Id., Channel Is., 38 m: 3, SBMNH 36066, Dago Bank, off San Pedro, Los Angeles Co.; 1, CAS 025200, Catalina Id., 79-140 m; 1, CAS 083447, Point Fermin, Los Angeles Co., 30 m; 3, LACM 65-3, S of Los Angeles Harbor, Los Angeles Co., 27 m; 2, LACM 65-2, SW of Point Fermin, Los Angeles Co., 29 m; 1, LACM 57-53, NW of Point Vincent Light, Los Angeles Co., 18 m; 24, LACM 72-91, off San Pedro, Los Angeles Co., 22 m; 1, CAS 083442, Cortez Bank, Channel Is., 12-27 m; 1, RNC, 264, Point Loma, San Diego Co., 15 m; 2, Hanselman Coll., Point Loma, San Diego Co., 15 m. Baja California, Mexico: 1, CAS 083446, Bahia de Todos Santos; 1, LACM 67-52, Arbolito, S side of Punta Banda, 15 m; 1, LACM 67-46, Puerto Santo Tomas, 13 m; 1, LACM 71-151, NE end of Isla Cedros, 5-12 m.

Distribution: *Tonicella venusta* occurs continuously from south-central Alaska to Isla Cedros, Baja California, Mexico (Fig. 37) at depths of 0-140 m. The northernmost record is Knight Island, Prince William Sound, Alaska (60°15'N, 147°44'W)(CAS 025595), the westernmost record is Chiniak Bay, Kodiak Id., Alaska (57°41'15"N, 152°24'00"W), and the southernmost record is Isla Cedros, Baja California (28°17'N, 115°8'W)(LACM 71-151).

Habitat and Ecology: *Tonicella venusta* lives on pebbles, cobbles, boulders, shells, and bedrock encrusted with coralline algae (*Lithothamnion* spp.) and bryozoans of unknown identity.

Fossil record: No fossil specimens of *Tonicella venusta* have been found. This is probably due to the small size and fragile nature of the valves.

Etymology: The name is Latin, and means lovely. The species is so named for the brilliant coloration of the valves in live animals, which are pink or lavender with blue or purple markings.

Remarks: Because of its small size and sandy girdle, *Tonicella venusta* has previously been confused with *Tonicella beringensis* (Yakovleva, 1952) [as *Tonicella*

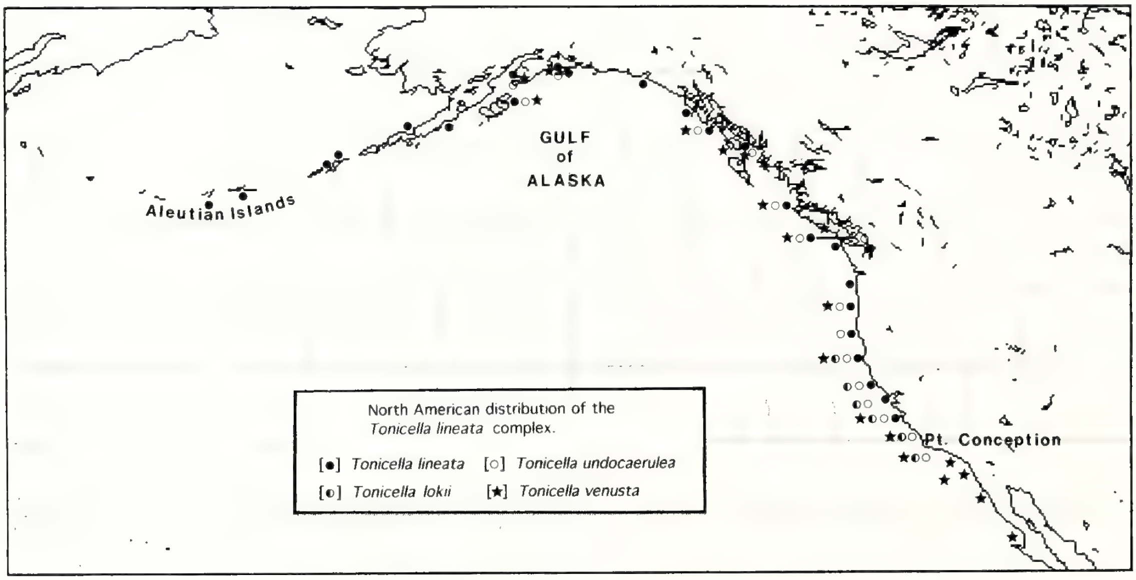

Fig. 37. Distribution of the *Tonicella lineata* complex.

rubra (Linnaeus, 1767), see discussion] from which it may be distinguished by its anterior mucro, concave post-mucronal slope and color pattern. *Tonicella venusta* has also been confused with *T. marmorea* (Fabricius, 1780), from which it is distinguished by the sandy girdle and the color pattern; with juveniles of *T. lineata*, from which it may be distinguished by the post-mucronal slope of the tail valve, sandy girdle and the color pattern; and with *Lepidochitona beanii* (Carpenter, 1855), from which it is distinguished by lacking large spicules in the girdle at the valve sutures.

DISCUSSION

The similarities in color pattern and habitat between the members of the *Tonicella lineata* complex are remarkable, and explain why the four taxa have been confused. Yet they differ in line pattern, post-mucronal slope of the tail valve and the morphology of the girdle elements and radula (Table 1). In addition to morphological characters of the valves, girdle elements and radula, these species also have distinct differences in the number of gills. In specimens of the same size, *Tonicella lokii* has slightly more numerous gills than *T. lineata*, and these two species both have more numerous gills than *T. undocaerulea* and *T. venusta*. *Tonicella venusta* has the fewest gills of the four (figure 38).

Berry (1917) was apparently misled by these similarities, as an examination of Berry's material from Forrester Island, Alaska revealed that he identified specimens of *Tonicella undocaerulea* as juveniles of *T. lineata*, but remarked that the "juveniles" were found off shore in 15-20 fathoms (27-36 m) and "adults" were found only on the shore. Additionally Berry identified both "*Tonicella rubra*" (see below) and *Tonicella venusta* as "*Tonicella rubra.*" Berry's specimens are preserved at the Santa Barbara Museum of Natural History.

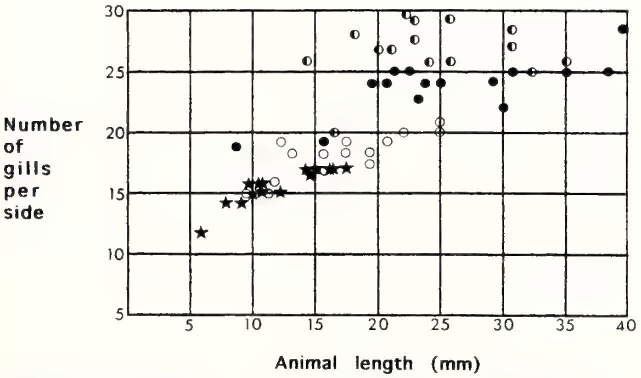

Fig. 38. Number of gills as a function of length (N = 15 specimens). *Tonicella lineata* [•]; *T. undocaerulae* [o]; *T. lokii* [◐]; *T. venusta* [★].

Table 1. Characters of taxa in the *Tonicella lineata* species complex.

Taxa/Character	*T. lineata*	*T. undocaerulea*	*T. lokii*	*T. venusta*
Dorsal girdle scales	ribbed	smooth w/ groove	smooth	rotund w/ apical pleats
Dimensions of scales (μm)	40 x 18	20 x 10	22 x 12	30 x 18
Central tooth of radula	narrow/ elongated	broad/ spoon-shaped	narrow/ spoon-shaped	narrow/ cupped head
Major lateral teeth	rounded/w 1-2 notches	tridentate	rounded/w 1 notch	tridentate
Brown lines on head valve	yes	no	yes	no
Head valve line pattern	gothic arch	zigzag	zigzag	zigzag
Flammules on pleural areas	no	no	no	yes
Post-mucronal slope of tail valve	+ / - straight	variable	concave	concave

The identity of *Tonicella lineata* var. *fusca* (von Middendorff, 1847) is questionable, as the type is lost (Sirenko, pers. comm., 1997), however the name, *fusca* (from the Latin *fuscus*) means dark or dusky, and most likely refers here to the dark, delineated form of this species, which is not uncommon throughout most of it's range, and which is particularly common from southeastern Alaska to Puget Sound, Washington. The type came from Sitka (Old Sitka). An example of this variation is illustrated in figure 33.

This report brings the total number of species in the genus *Tonicella* in the northeastern region of the Pacific Ocean to six. These include: *Tonicella lineata* (Wood, 1815), *Tonicella undocaerulea* Sirenko, 1973, *Tonicella lokii* Clark, *spec. nov.*, *Tonicella venusta* Clark, *spec. nov.*, *Tonicella insignis* (Reeve, 1847) and *Tonicella submarmorea* (Middendorff, 1846) [here considered distinct from *Tonicella marmorea* (Fabricius, 1780) on the basis of valve and radula morphology]. I follow Yakovleva (1952) and Sirenko (1974) in considering *Tonicella rubra* (of authors) from the Pacific as *T. beringensis* Yakovleva, 1952. Both of these species will soon be reclassified in another genus (Sirenko, pers. comm.).

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Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding

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Abstract: The effects of controlled feeding versus starvation during quarantine on mantle tissue glycogen concentration (represented as milligrams glycogen per gram of mantle tissue ± SD) of two freshwater mussel species were compared. Starved individuals were not provided with supplemental food during quarantine, while fed specimens were provided with 10^5 algal cells/ml, twice per day. Initial mean glycogen levels for *Amblema plicata* (Say, 1817) (9.4 ± 2.4 mg/g) and *Quadrula pustulosa* (I. Lea, 1831) (7.9 ± 1.8 mg/g) collected from Ohio River Mile (ORM) 175.5 in July 1997 were not significantly different ($p > 0.3$) from mean glycogen levels of *A. plicata* (8.1 ± 4.2 mg/g) and *Q. pustulosa* (6.2 ± 2.9 mg/g) collected from the same site in July 1996. Initial glycogen concentrations of quarantined mussels, therefore, were similar in both the starved and fed groups. After seven days of feeding in quarantine, mean glycogen levels of *A. plicata* (12.3 ± 2.3 mg/g) and *Q. pustulosa* (7.1 ± 3.7 mg/g) did not change significantly ($p > 0.1$) relative to wild-caught specimens, and were significantly larger ($p < 0.05$) than mean glycogen levels of starved individuals (3.6 ± 1.8 mg/g and 3.5 ± 2.3 mg/g, respectively). Similarly, mean glycogen levels of *A. plicata* and *Q. pustulosa* after 14 days (8.1 ± 3.3 mg/g and 7.7 ± 3.3 mg/g, respectively) and 30 days (9.9 ± 4.8 mg/g and 8.4 ± 2.7 mg/g, respectively) of feeding were significantly larger ($p < 0.01$) than mean glycogen levels of starved specimens after 14 days (3.27 ± 1.74 mg/g and 5.37 ± 3.06 mg/g, respectively) and 30 days (1.2 ± 0.5 mg/g and 1.9 ± 1.4 mg/g, respectively). Adequate feeding of unionids in quarantine is essential to maintain animals in a condition that will increase the likelihood of survival following relocation.

Key Words: glycogen, zebra mussels, Ohio River, quarantine, Dreissenidae

Relocation has been used widely as a management tool to re-establish or assist in the recovery of declining populations of terrestrial and aquatic organisms (Griffith *et al.*, 1989; Cope and Waller, 1995). In response to recent declines in the unionid fauna of North America (Williams *et al.*, 1993), relocation is being tested as a management technique to (1) re-introduce extirpated populations, (2) remove unionids from project construction zones, (3) augment populations to increase genetic diversity, and (4) salvage unionids from zebra mussel-infested areas (Cope and Waller, 1995). The ultimate goal of any relocation project is to establish a self-sustaining population of the target organism, and several authors have developed a list of factors that should extend population persistence after relocation, including (1) the presence of suitable habitat and refugia, (2) the release of large founder groups with high genetic diversity, (3) low levels of competition, and (4) high rates of population growth (Griffith *et al.*, 1989; Cope and Waller, 1995). Little attention, however, has been given to the biochemical and physiological condition of an organism prior to relocation and its influence on survival and the ability to develop reproductively viable populations after relocation.

Condition, as defined by Mann (1978: 490), is the "ability of an animal to withstand an adverse environmental stress, be this physical, chemical or biological." Glycogen is the primary energy store in bivalves (Bayne, 1976; Gabbott, 1983), and the relative amount of glycogen stored in bivalve tissues is considered a good indicator of body condition (Galtsoff, 1964; Walne, 1970). Significant reductions in unionid glycogen stores prior to and during relocation, therefore, could greatly reduce their ability to cope with natural stressors present in the new environment.

Collection, handling, aerial exposure, abrupt temperature changes, holding, and transport of unionids during relocation can result in varying degrees of stress, depending on the length of exposure, that can in turn adversely affect body condition (Cope and Waller, 1995). Relocation of unionids to avoid the adverse effects of bridge construction, for example, could have little or no effect on condition if individual specimens are removed from their natural habitat for short time periods (several hours or days). The presence of zebra mussels, however, has been shown to cause decreased glycogen reserves in unionids (Haag *et al.*, 1993). In addition, zebra mussel-infested unionids presently require a 30-day quarantine period to ensure that zebra mussel adults and juveniles are removed prior to relocation (J. Clayton, pers. comm.). In addition to the stress of relocation, unionids can experience nutritive stress in quarantine because little or no information exists on their nutritional requirements (Gatenby *et al.*, 1997). Under laboratory or hatchery conditions, bivalve energy stores have been shown to decline without proper feeding (Calvin, 1931; Pora *et al.*, 1969; Bayne and Thompson, 1970; Gabbott and Walker, 1971), and recent studies reveal that unionid glycogen stores can decline as much as 80% after only 30 days of starvation in quarantine (Patterson *et al.*, 1997). Consequently, the development of a feeding regime for mussels held in quarantine could be a critical link in the success of future relocation projects. The objectives of this experiment were to (1) monitor the glycogen levels of unionids during controlled feeding in quarantine, and (2) compare these results to reported changes in unionid glycogen levels during starvation in quarantine, as reported by Patterson *et al.* (1997).

METHODS

On 20 July 1997, ten specimens each of *Amblema plicata* (Say, 1817) and *Quadrula pustulosa* (I. Lea, 1831) were collected from Ohio River Mile (ORM) 175.5 near Parkersburg, West Virginia. Mussels were removed from the shell and placed in 95% ethanol for the determination of initial glycogen levels. Additional specimens of *A. plicata* and *Q. pustulosa* (N = 200 and 80, respectively) were collected from ORM 175.5 for placement in individual quarantine tanks. Unionids salvaged from zebra mussel-infested waters were thoroughly scrubbed to remove zebra mussels. Cleaned unionids were then hand-inspected before being placed in aerated quarantine tanks without substratum for a minimum of 30 days. During this 30-day period, water temperatures were maintained around 20°C to allow juvenile zebra mussels missed during the scrubbing procedure to become visible. At the end of 30 days, individual unionids were inspected under 10X magnification to ensure the absence of zebra mussels prior to relocation. Ten specimens of *A. plicata* and *Q. pustulosa* were sacrificed after seven, 14, and 30 days of quarantine, and preserved in 95% ethanol for subsequent glycogen analysis. After the experiment, all remaining specimens were returned to the Ohio River.

In 1997, unionids were fed cultures of the chlorophyte, *Neochloris oleoabundans* Chantanachat and Bold, 1962. This species was chosen because previous experiments indicated that juvenile unionids readily ingest and assimilate this alga (Gatenby *et al.*, 1997). Initial stock cultures of *N. oleoabundans* were grown in Bold's Basal Medium (Nichols, 1973) under continuous cool white fluorescent light (photon flux: 60-100 $\mu E/m^2/s$) at 20°C. Stock cultures were then transferred to the quarantine facility and used to inoculate a 20 l carboy containing Fritz F2 algal medium (Fritz Aquaculture, Mesquite, Texas). When cell densities in the carboy reached 10^6 cells/ml, 7 l aliquots were used to inoculate three 250 l algal culture tanks (Aquatic Ecosystems, Inc., Apopka, Florida), that also were fertilized with Fritz F2 algal medium, aerated, and placed outside the quarantine facility in direct sunlight. Culture tanks were placed outside because algal cultures continually failed inside the quarantine facility, possibly due to insufficient light or high water temperatures. Algal cell densities in the 250 l culture tanks reached 10^6 cells/ml in *ca.* 4 days. Water samples from the culture tanks were collected daily and fixed with acid Lugol's solution (Saraceni and Ruggiu, 1969) for enumeration and identification of the algae. Water samples from the Ohio River in 1996 and 1997 showed that algal cell densities ranged from 10^4 - 10^5/cells/ml during the summer (Parker *et al.*, 1998), thus unionids in quarantine were fed ca. 10^5 cells/ml twice per day, at 8:00 AM and 5:00 PM. Each day, 75% of the water in the quarantine tanks was drained; fresh water and food were added, and vigorous aeration was applied to maintain algal cells in suspension.

The glycogen content of all preserved specimens was determined from mantle tissue using the technique of Keppler and Decker (1974) as described in Patterson *et al.* (1997). Mean glycogen levels were expressed in milligrams of glycogen per gram of preserved mantle tissue ± standard deviation (SD). It should be noted that preserved tissue weights overestimate dry weights and underestimate wet tissue weights because 95% ethanol dehydrates tissue. Dehydration by 95% ethanol also reduces error that can result from changes in tissue water levels during stress. Mean glycogen levels were compared using ANOVA and, if significant differences were detected, the Scheffe F-test was used to determine the statistical significance of individual treatments.

RESULTS AND DISCUSSION

During the first 14 d of quarantine, *Neochloris oleoabundans* comprised > 95% of the algae in the 250-l culture containers. Because culture tanks were maintained outside the quarantine facility, cultures were contaminated with low densities of two green algae, *Scenedesmus* sp. and *Ankistrodesmus* sp. Once cultures were contaminated, densities of these contaminants continued to increase. After 30 d, the algal community in the culture containers had changed significantly, with *Scenedesmus* and

Ankistrodesmus comprising *ca.* 40% of the available algae, and *N. oleoabundans* comprising the remaining 60%. Despite changes in the algal community, cell densities remained at 10^6 cells/ml throughout the experiment.

Both the fed and starved treatments had some intial mortality likely as a result of collection and handling, however, no mortality occurred during the remainder of the quarantine period. Mean glycogen levels of *Amblema plicata* and *Quadrula pustulosa* during controlled feeding in quarantine remained the same, whereas mean glycogen levels declined in a previous starvation experiment by Patterson *et al.* (1997) (Fig. 1). Initial mean glycogen levels for *A. plicata* (9.4 ± 2.4 mg/g) and *Q. pustulosa* (7.9 ± 1.8 mg/g) collected from ORM 175.5 in July 1997 were not significantly different ($p > 0.3$) from the mean glycogen levels of *A. plicata* (8.1 ± 4.2 mg/g) and *Q. pustulosa* (6.2 ± 2.9 mg/g) collected from the same site in July 1996 (Patterson *et al.*, 1997). Glycogen stores of unionids entering quarantine, therefore, were similar in both the starvation and controlled feeding experiments. After seven days of feeding in quarantine, mean glycogen levels of *A. plicata* (12.3 ± 2.3 mg/g) and *Q. pustulosa* (7.1 ± 3.7 mg/g) did not change significantly ($p > 0.1$) relative to wild-caught specimens, and were significantly larger ($p < 0.05$) than mean glycogen levels of starved individuals (3.6 ± 1.8 mg/g and 3.5 ± 2.3 mg/g, respectively). Similarly, mean glycogen levels of *A. plicata* and *Q. pustulosa* after 14 days (8.1 ± 3.3 mg/g and 7.7 ± 3.3 mg/g, respectively) and 30 days (9.9 ± 4.8 mg/g and 8.4 ± 2.7 mg/g, respectively) of feeding were significantly larger ($p < 0.01$) than mean glycogen levels of starved specimens after 14 days (3.27 ± 1.74 mg/g and 5.37 ± 3.06 mg/g, respectively) and 30 days (1.2 ± 0.5 mg/g and 1.9 ± 1.4 mg/g, respectively).

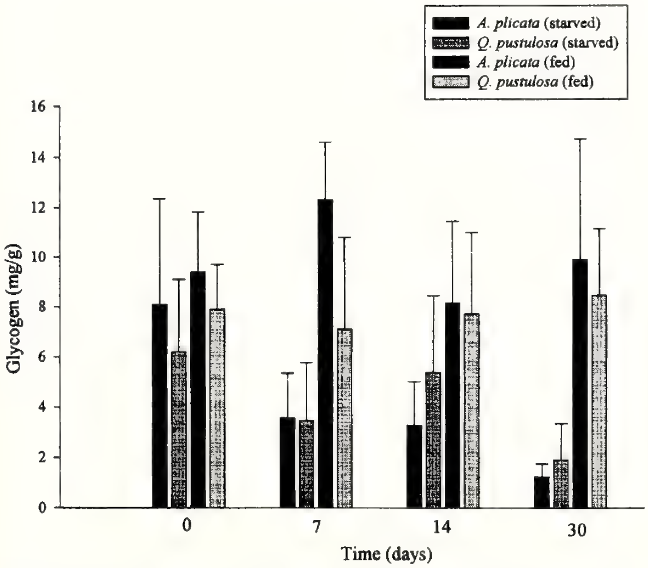

Fig. 1. Glycogen levels of *Amblema plicata* and *Quadrula pustulosa* at 0, 7, 14, and 30 d of starvation and controlled feeding in quarantine (N = 10/sampling period).

Glycogen, the primary energy reserve in bivalves, drives many important physiological processes and can be used to endure short-term exposure to anoxia, emersion, or reduced food supplies (Bayne, 1976; Gabbott, 1983; Bayne *et al.*, 1985; Hummel *et al.*, 1988). Although exposure to anoxia and emersion can be limited if unionids are relocated to suitable habitat, unionids will likely experience short-term, localized shifts in food abundance and long-term food shortages during the winter months. Normally, by accumulating glycogen when food is abundant, bivalves are able to withstand these food shortages (Gabbott, 1983; Hummel *et al.*, 1988). However, unionid survival after relocation could be greatly reduced if glycogen stores are depleted in quarantine and do not recover prior to the onset of winter. Regardless of effects on survival, decreased glycogen levels in adult bivalves also can have sublethal effects including reduced fecundity and reduced growth rates of developing offspring (Bayne, 1972; Helm *et al.*, 1973; Bayne *et al.*, 1975). Thus, the provision of adequate food resources for unionids in quarantine is pertinent to maintaining glycogen levels and enabling mussels to develop reproductively viable populations after relocation.

Results from this study indicate that relatively high cell densities of the green alga, *Neochloris oleoabundans*, in combination with *Scenedesmus* and *Ankistrodesmus*, is an adequate food resource for the maintenance of unionid glycogen stores in the short term (30 d). Currently, no information exists on the ability of unionids to digest and assimilate *Scenedesmus* and *Ankistrodesmus*, but recent studies show that unionids assimilate *Neochloris oleoabundans* with relatively high efficiency (> 50%, M. Patterson, unpub. data). Additional studies to determine which algal species are readily digested and assimilated by unionids are critical, because some algal species might not be readily digested and assimilated by certain bivalve species (Peirson, 1983). Regardless of the digestibility of a particular algal food resource, large amounts of algae will be required if management agencies hope to relocate large numbers of native mussels away from zebra mussel-infested areas. In this study, the quarantine of 300 mussels required constant culture of 750 l of living algae. Discovering ways to shorten the quarantine period would make the production of algae more feasible and decrease the time that unionids must be maintained in captivity, outside their natural habitat. Studies dealing with more efficient methods of removing zebra mussels from the shells of unionids could prove to be the best avenue for reducing the quarantine period. Ultimately, a short quarantine period along with the provision of food will improve the body condition of unionids and improve the success of future relocations.

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Variation in glycogen concentrations within mantle and foot tissue in *Amblema plicata plicata*: Implications for tissue biopsy sampling

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Abstract: With the development of techniques to non-lethally biopsy tissue from unionids, a new method is available to measure changes in biochemical, contaminant, and genetic constituents in this imperiled faunal group. However, before its widespread application, information on the variability of biochemical components within and among tissues needs to be evaluated. We measured glycogen concentrations in foot and mantle tissue in *Amblema plicata plicata* (Say, 1817) to determine if glycogen was evenly distributed within and between tissues and to determine which tissue might be more responsive to the stress associated with relocating mussels. Glycogen was measured in two groups of mussels: those sampled from their native environment (undisturbed mussels) and quickly frozen for analysis and those relocated into an artificial pond (relocated mussels) for 24 months before analysis. In both undisturbed and relocated mussels, glycogen concentrations were evenly distributed within foot, but not within mantle tissue. In mantle tissue, concentrations of glycogen varied about 2-fold among sections. In addition, glycogen varied significantly between tissues in undisturbed mussels, but not in relocated mussels. Twenty-four months after relocation, glycogen concentrations had declined by 80% in mantle tissue and by 56% in foot tissue relative to the undisturbed mussels. These data indicate that representative biopsy samples can be obtained from foot tissue, but not mantle tissue. We hypothesize that mantle tissue could be more responsive to the stress of relocation due to its high metabolic activity associated with shell formation.

Key Words: glycogen, tissue variation, *Amblema plicata*, biopsy, relocation

With the development of techniques to obtain biopsy samples from mantle (Berg *et al.*, 1995; Byrne and Vesk, 1997) and foot tissue (Naimo *et al.*, 1998) in unionids, a new tool has emerged to measure the biochemical, contaminant, and genetic constituents in this imperiled faunal group. These techniques permit the measurement of indices of an organism's physiological condition without adversely affecting survival. Recently, biopsy samples have been removed from four unionid species (Berg *et al.*, 1995; Byrne and Vesk, 1997; Naimo *et al.*, 1998) and survival of biopsied and non-biopsied mussels has been similar for up to 19 months following biopsy. Furthermore, because of their nondestructive nature, these techniques have potential for use on endangered, threatened, or otherwise sensitive populations. However, possible variation in concentration of a given constituent within a tissue type is unknown. Because only a small mass of biopsied tissue is taken (about 10 to 40 mg wet weight), the representativeness of this sample must be determined.

In the past few years, considerable effort has been invested into the relocation of unionids as a conservation strategy, principally in response to the threat of the exotic zebra mussel (Cummings *et al.*, 1997). As a consequence, numerous researchers are developing methods for relocation and for evaluating the success of relocations (Dunn and Layzer, 1997; Dunn and Sietman, 1997; Patterson *et al.*, 1997). However, because of the long life span of mussels, traditional measures of condition, such as changes in shell length or tissue weight, are often inappropriate for short-term observations. Thus, the identification of sublethal indicators of stress in unionids could provide valuable data on physiological condition that precedes measurable changes in survival.

We measured glycogen, because it is the principal storage form of carbohydrates in many aquatic invertebrates (Stetten and Stetten, 1960; De Zwaan and Zandee, 1972; Hummel *et al.*, 1989) and it has been used as an indicator of the energetic status of individual mussels (Holopainen, 1987; Hemelraad *et al.*, 1990). Alterations in glycogen concentrations in unionids can be observed long before changes in either growth or survival are observed. For example, 3-month exposure to zebra mussels significantly reduced glycogen concentrations in *Amblema plicata* but did not adversely affect survival (Haag *et al.*, 1993). Furthermore, measurement of glycogen concentration is a good indicator of nutritional stress in unionids during quarantine periods before relocation (Patterson *et al.*, 1997).

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Our goal was to determine the representativeness of biopsied tissue for the analysis of glycogen concentrations in foot and mantle tissue in *Amblema plicata plicata* (Say, 1817). Our objectives were (1) to determine if glycogen concentrations were evenly distributed within mantle and foot tissue; (2) to determine if glycogen concentrations varied between tissues; and (3) to qualitatively determine which tissue might be more responsive to the stress associated with relocating mussels from their native environment into an artificial environment.

METHODS

TISSUE SAMPLING

To estimate the distribution of glycogen in foot and mantle tissue in *Amblema plicata plicata*, we relocated individuals from Pool 9 of the Upper Mississippi River in May 1995, and placed them into mesh bags in a 0.04 hectare earthen pond. The pond was filled with well water (retention time of about 1 week) and was continuously aerated. No supplemental food was added to the pond during the study. After 24 months (June 1997), 5 individuals were removed, and mantle and foot tissue were dissected and subsequently frozen at -84°C until analysis for glycogen. To determine if the relocation altered the distribution of glycogen within and between tissues, we also determined the distribution of glycogen in undisturbed *Amblema plicata plicata* that were removed directly from the Upper Mississippi River in May 1997, but not subjected to relocation.

In both groups (relocated and undisturbed), we sacrificed 5 individuals (shell length 80-90 mm) for determination of glycogen. All unionids were free of zebra mussels. In each individual, the mantle and foot tissue were dissected into three sections. In mantle, the three 5-mm sections extended ventral (section A) to dorsal (section C) and in foot tissue, the three 15-mm sections extended anterior (section A) to posterior (section C; Fig. 1). All foot sections were removed from the ventral margin to exclude reproductive tissue from the sample. Within each section, we randomly removed five 5-mm^2 samples, mean 10.1 ± 0.1 mg wet weight in both foot and mantle tissue.

Because glycogen is frequently reported on a wet-weight basis, spatial differences in water content within a given tissue may bias estimates of glycogen concentrations. To determine if water content varied within a tissue, we measured the percent water in each section of foot and

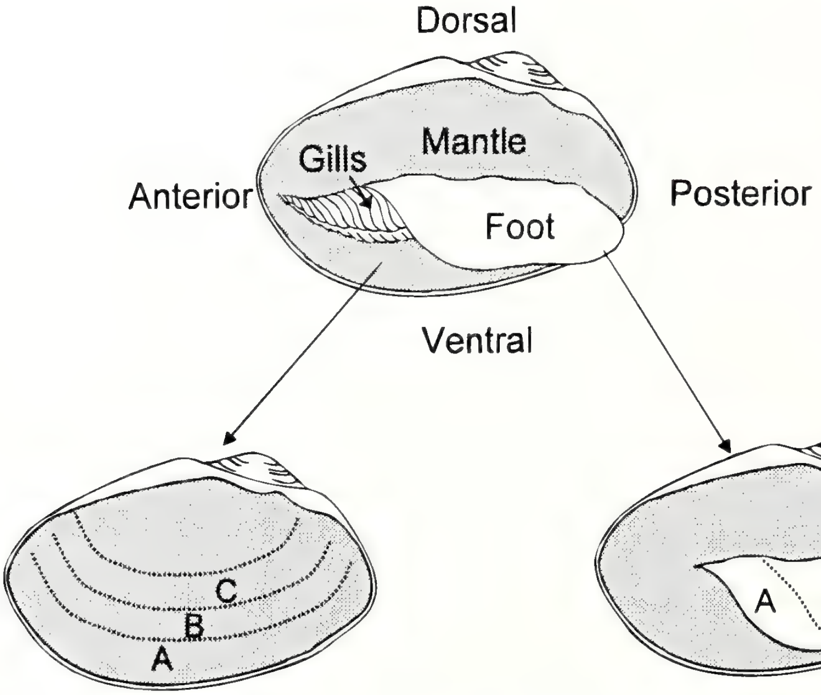

Fig. 1. Sample locations for foot and mantle tissue in *Amblema plicata plicata*. In the mantle, the three 5-mm sections extended ventral (section A) to dorsal (section C) and in the foot, the three 15-mm sections extended from anterior (section A) to posterior (section C).

(Hemminga *et al.*, 1985). Similarly, in the marine mussel *Mytilus edulis* (Linnaeus, 1758), the hepatopancreas and the mantle have been identified as the primary storage organs for glycogen (Bayne, 1973a, b; Zaba *et al.*, 1981). However, comparisons of glycogen concentrations between mantle tissue in freshwater and marine mussels should be made cautiously because mantle tissue in *Mytilus* is also the site for gonad development (Zaba *et al.*, 1981). Although we observed elevated concentrations of glycogen in mantle tissue, relative to foot tissue, in undisturbed *Amblema plicata plicata* this does not necessarily suggest that mantle tissue is a storage site for glycogen. Because the mass of foot tissue likely exceeds that of mantle tissue, the mass of glycogen in the foot could easily surpass that in the mantle.

The consistent reduction in glycogen concentrations in the relocated mussels, relative to the undisturbed mussels, suggests that the relocated mussels were not obtaining adequate nutritional requirements in this artificial environment. For example, mean concentrations of chlorophyll *a* (± 1 SEM) in the pond averaged 11 ± 4 µg/L (n=11) during April through November, whereas concentrations in the river averaged 51 ± 9 µg/L (n=15) during this same period. Furthermore, the magnitude of the reduction in glycogen was substantial--80% in mantle tissue and 56% in foot tissue. Similarly, Patterson *et al.* (1997) reported a 67% reduction in glycogen concentrations in mantle tissue in *Amblema plicata* from a site in the Ohio River heavily infested by zebra mussels (3,600 zebra mussels/m^2) relative to glycogen concentrations in mussels from a lightly infested site (0.3 zebra mussel/m^2). These data suggest that the relocation of unionids into refugia could be unsuccessful if the new environment does not contain adequate nutritional resources. In addition, even if adequate food resources are available, mussels must be physiologically-capable of obtaining and processing the available food.

Our criteria for selecting which tissue to biopsy for biochemical analysis were (1) within section variation in glycogen was minimal; (2) glycogen concentrations were similar across sections; and (3) it would be responsive to stress (in this case, relocation to an artificial pond). Using these criteria, the measurement of glycogen in foot tissue meets all three criteria. While the magnitude of the responsiveness to stress was greater in mantle tissue than in foot tissue, glycogen concentrations were not evenly distributed and were highly variable in mantle tissue. However, this should not preclude the use of mantle tissue, it just suggests a need for consistency in the location of the biopsied sample. In addition, the water content in a given section should be determined and used when reporting glycogen on a dry-weight basis in mantle tissue. If the mantle tissue is sampled, we recommend sampling the 5-mm section closest to the shell margin (section A) because it may be the most metabolically active and the easiest section to biopsy.

Although this study was conducted on a few individuals of *Amblema plicata plicata*, our preliminary data suggest that glycogen concentrations vary between and within certain tissues, and between relocated and undisturbed mussels. However, the applicability of these data to other species at other times of the year remains unknown. Future studies need to examine the influence of confounding factors, such as reproductive status, seasonal trends, and differences between sexes, on the distribution and utilization of glycogen in several unionid species.

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Recruitment in a freshwater unionid (Mollusca: Bivalvia) community downstream of Cave Run Lake in the Licking River, Kentucky

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Abstract: Unionids, fish, and glochidia were collected to determine why recruitment had ceased or had been dramatically decreased in a speciose unionid community in the Licking River at Moores Ferry, Kentucky, 35.4 km downstream of Cave Run Lake. Only six unionid glochidia were collected with drift nets, and only six fish collected had infestations of glochidia. A small percentage (10.1%) of the unionids observed had their gills modified as marsupia. An analysis of water temperature and discharge indicated no significant difference in average monthly discharge ($p > 0.05$) and a significant decrease in temperature ($p < 0.05$) between pre- and post-impoundment periods. Average monthly discharge and temperature may not be as biologically important as the spikes of discharge and corresponding sudden decreases in temperature that are caused by releases of hypolimnionic water from the reservoir.

Key Words: Unionidae, recruitment, gametogenesis, impoundments, Kentucky

North America has the richest unionoidean (mussel) (Bivalvia: Unionidae) fauna in the world. This fauna has disproportionately more endangered, threatened, and special concern taxa than all the groups of terrestrial organisms. Only 70 of the 297 taxa known from the United States are considered stable (Williams *et al.*, 1993). Of the freshwater unionid taxa recognized from the United States and Canada, 35% (103 taxa) are known to occur, or have occurred in Kentucky, which ranks this state third in faunal richness behind Tennessee and Alabama (Cicerello *et al.*, 1991). Human activities in the Commonwealth have severely impacted unionid populations during the last 200 years, making this group of organisms the most endangered in the state (Cicerello *et al.*, 1991).

Freshwater mussels are impacted by anthropogenic factors such as the additions of toxic substances into aquatic systems, sedimentation, habitat destruction, loss of their host fish(es), introduced species, and commercial harvesting (van der Schalie and van der Schalie, 1950; Fuller, 1980; Bogan, 1993; Williams *et al.*, 1993). The construction of dams along the course of several rivers in North America has resulted not only in the loss of taxa, but also of whole beds (*e. g.*, van der Schalie and van der Schalie, 1950; Bates, 1962; Miller *et al.*, 1984; Miller *et al.*, 1992; Williams *et al.*, 1992; Layzer *et al.*, 1993; Sickel and Chandler, 1996). One of the most severe and perplexing problems facing freshwater mussels is the documented loss of recent recruitment (reproduction) in unionid communities that were previously thought to be healthy.

Recent research hypothesized that recruitment had ceased or had been dramatically decreased in a diverse unionid community in the Licking River at Moores Ferry, Kentucky (Kane, 1990; Smathers, 1990). The present study was an attempt to determine if recruitment had ceased or had been decreased in this diverse unionid community, and if so, to determine where in the life cycle reproduction was breaking down. This study was also an attempt to determine what effects, if any, the hypolimnionic discharges from Cave Run Lake were having on the mussels in the bed at Moores Ferry. It was hypothesized that the loss of recruitment in this bed was either directly or indirectly the result of the dam, located approximately 35.4 km upstream, which has altered both the natural temperature and flow regimes of the river.

METHODOLOGY

STUDY AREA

The Licking River is a sixth order tributary to the Ohio River, originating on the Unglaciated Allegheny Plateau in the Appalachian Province of eastern Kentucky. The river flows through an extremely variable topography in a northwesterly direction through the Blue Grass region of the Commonwealth for 496 km until its confluence with the Ohio River near Covington, Kentucky, at Ohio River km 757.2 (Harker *et al.*, 1979, Hannan, *et al.*, 1982, Burr and Warren, 1986). This drainage encompasses approximately 10% of the Commonwealth (9601 km^2) and covers all or a portion of 21 counties (Harker *et al.*, 1979).

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In 1974 the Licking River was impounded to form Cave Run Lake, a warm oligotrophic lake (Clinger, 1974). The lake has a surface area of 3347 ha that inundates 61 km of the mainstem and the lower reaches of several small tributaries (Burr and Warren, 1986). The Licking River drainage has a speciose unionid fauna with 53 taxa, over half of the state's native mussel fauna, historically existing in the drainage (Cicerello *et al.*, 1991). A recent survey below the reservoir indicated that 50 taxa still reside in that portion of the river (Laudermilk, 1993).

Moores Ferry, Kentucky, is a ford approximately 35.4 km downstream from Cave Run Lake (Fig. 1). The watershed at this site is utilized mainly for agriculture. Discharge at this site is affected by releases of water from Cave Run Lake, which at times causes drastic water level fluctuations. Substrata consisted mainly of cobble, gravel, rubble, and some boulders with intermixed sandy areas (Smathers, 1990). The unionid community at this site had a rich assemblage of unionid species, with 35 known present or historical taxa (Smathers, 1990; Laudermilk, 1993).

SAMPLING AND LABORATORY PROCEDURES

Five collections of glochidia, fish, and unionids were made from July through October 1995. High water conditions prevented collections during Spring 1996. For each collection period a drift net was haphazardly placed in the bed to collect glochidia. After one hour, the contents of the drift net were removed and preserved in 70% ethanol and returned to the laboratory. Drift net collections were examined using cross-polarized light microscopy (Johnson, 1995), with a portion of the sample being delivered into a watch-glass with a gridded bottom. Each square in the grid was then systematically searched under cross-polarized light (10-20X) for glochidia, which were counted and removed along with any juvenile *Corbicula fluminea* (Müller, 1774).

Fish were collected for one hour using a minnow seine. All fish retained were initially preserved in a 10% formalin solution and then transferred to 70% ethanol in the laboratory for final preservation. Following sorting and identification, the fins and scales of each individual were examined under a dissecting microscope (10-30X) for attached glochidia. The opercular flaps were removed, and each gill arch was carefully examined under a dissecting microscope for attached glochidia (Bruenderman and Neves, 1993).

Unionids were collected by hand for one hour by snorkeling, or by wading with the use of water scopes. After identification, the shell of each unionid was carefully opened with a small screwdriver, and the gills examined for signs of gravidity. The species name was recorded and notes were made on the condition of the gills. Except for individuals of two target species retained for histological examination, all unionids were returned to the river. Three to five individuals of the two most common species in the bed at Moores Ferry (Smathers, 1990), *Actinonaias ligamentina* (Lamarck, 1819) and *Elliptio dilatata* (Rafinesque, 1820), were chosen for histological examination from each collecting period. These species represented both of the breeding regimes of freshwater mussels, they were both commonly encountered throughout their respective ranges (Oesch, 1995), and neither had any federal or state protection status in Kentucky (Kentucky State Nature Preserves Commission [KSNPC], 1996).

Individuals were prepared for histological examination by placing them into a 10% formalin solution, then transferring them to 70% ethanol in the laboratory. The valves were opened by cutting the adductor muscles and portions of the gonadal and gill tissues were removed and placed into either 70% ethanol or Bouins fixative. These were then dehydrated through a series of alcohols and embedded in paraffin (Humason, 1967). Sections were made at a thickness of 10 µm using an American Optical 820 Microtome. Slides were stained with Ehrlich's hematoxylin and eosin was used as the counterstain (Humason, 1967). Sections were mounted with Permount. The sections were then examined under a compound microscope (400-430X) to determine a sex ratio for both species; to determine if gametogenesis was occurring, and, if so, to try to quantify it; and to determine the contents of the marsupia. All drift net, fish, and unionid collections were deposited in the Branley A. Branson Museum of Zoology, Eastern Kentucky University (EKU).

Five cell types of spermatogenesis (Garner, 1993) were used to determine the stage of gametogenesis in males. Stage 1 males were those that had only spermatogonia present in their acini, and Stage 5 males had mature spermatozoa present. Stages 2, 3, and 4 corresponded respectively to sperm morulae, spermatocytes, and spermatids being present in the acini. Three cell types of oogenesis (McMurray, 1997) were used to determine the stage of gametogenesis in female *Elliptio dilatata*. Stage 1 females were those with oogonia as the dominant cell type in their alveoli, Stage 2 were those with oocytes dominant, and Stage 3 were those with mature ova dominant.

Marsupia were classified according to their contents as being empty (EM), or containing mature glochidia (MG), early embryos (EE), or advanced embryos (AE) (Garner, 1993). In the case of known females that did not have their gill tissues examined, the marsupia were consid-

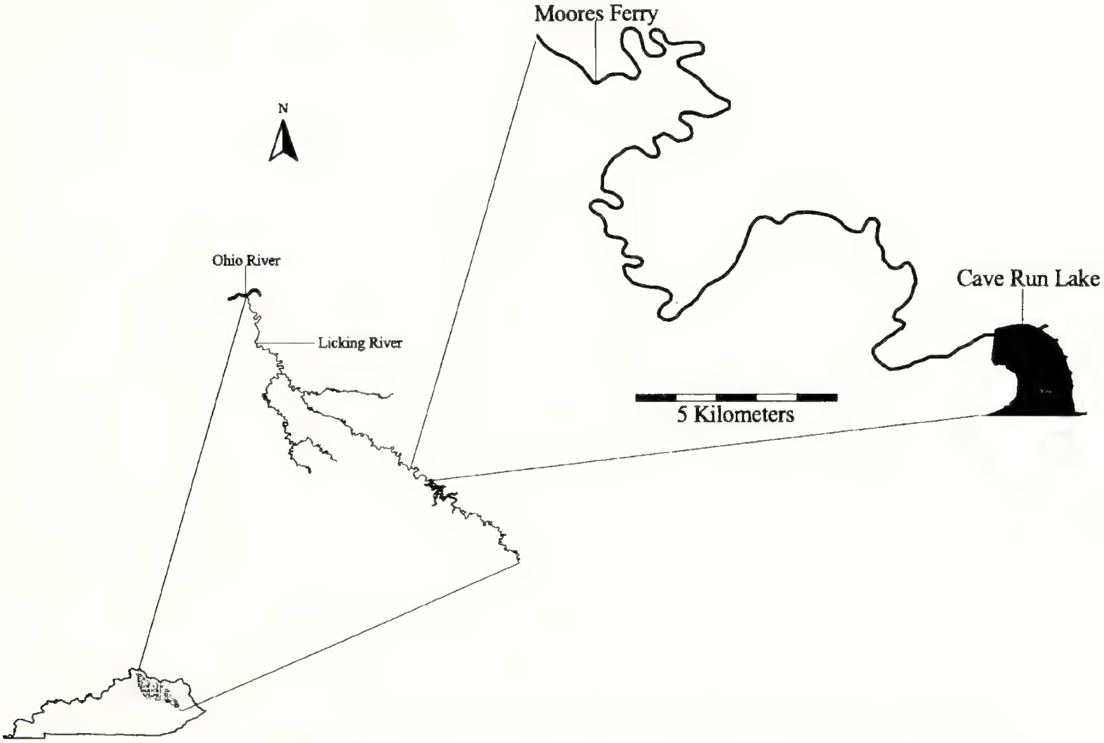

Fig. 1. Location of the Moores Ferry study site. Inset shows the location of the Licking River drainage in Kentucky.

ered to be empty since sections were made of any gill that showed signs of gravidity. Marsupia with mature glochidia were those that had glochidial shells and the single adductor muscle visible in the section.

Temperature and discharge data were obtained from the United Stages Geological Survey (USGS) for the Licking River gauging station at Farmers, Kentucky, which is just upstream of Moores Ferry. Data were obtained for a pre-impoundment (1966-1970) and a post-impoundment (1991-1994) period. Daily temperature and discharge data from the months of May through September for each of these years, with the exception of 1994 (May-June), were analyzed to determine if any correlation existed between discharge and water temperature.

RESULTS AND DISCUSSION

A total of only six unionid glochidia were collected using drift nets, compared to 185 juvenile *Corbicula fluminea*. The large number of juvenile *C. fluminea* was largely the result of a single collection of 127 individuals. Drift net collections were made between 1000 and 1700 hours (EST), which corresponds to the period when glochidial densities should have been at their highest (Kitchell, 1985). The high number of *C. fluminea* present may impact juvenile unionids through resource competition (Neves and Widlak, 1987).

Only 11.3% of the 399 fish collected were suitable hosts for unionids in the bed (Watters, 1994). Of the fish collected only six specimens had attached glochidia: one *Micropterus punctulatus* (Rafinesque, 1819); three *Percina copelandi* (Jordan, 1877); one *P. evides* (Jordan and Copeland, 1877); and one *P. oxyrhyncha* (Hubbs and Raney, 1939). Even though a small fish may successfully carry several hundred glochidia (Lefevre and Curtis, 1910), low infestation rates appear to be common (Kitchell, 1985; Neves and Widlak, 1988; Bruenderman and Neves, 1993; Weiss and Layzer, 1995). The attachment of glochidia to their hosts is dependent upon several factors such as infestations of hosts by copepod parasites (Wilson, 1916), age of the host, immunity caused by previous infestations (Parker *et al.*, 1984), and water temperature (Matteson, 1948).

A total of 288 unionids were collected from Moores Ferry (Table 1). Of these, only 10.1% (29 individuals) had their gills modified as marsupia, indicating an 8.93:1 male to female ratio if modified gills are taken to represent females. Marsupial condition is probably not a true representation of the actual male to female ratios, but most unionids are not sexually dimorphic (McMahon, 1991), and the only way to determine the sex of an individual without using histological techniques is to examine the gills for signs of gravidity. However, it would still be expected that at least 50% of the individuals collected would have had

Table 1. Unionids with and without gills modified as marsupia from the Licking River at Moores Ferry, Kentucky.

Taxa	With Modified Gills	Without Modified Gills	Totals
Actinonaias ligamentina (Lamarck, 1819)	20	135	155
Alasmidonta marginata Say, 1818	0	0	0
Amblema plicata (Say, 1817)	0	45	45
Cyclonaias tuberculata (Rafinesque, 1820)	0	0	0
Elliptio dilatata (Rafinesque, 1820)	0	18	18
Fusconaia flava (Rafinesque, 1820)	0	2	2
Lampsilis cardium Rafinesque, 1820	2	6	8
Lasmigona complanata (Barnes, 1823)	0	0	0
L. costata (Rafinesque, 1820)	0	6	6
Leptodea fragilis (Rafinesque, 1820)	0	1	1
Megalonaias nervosa (Rafinesque, 1820)	0	9	9
Obliquaria reflexa Rafinesque, 1820	0	0	0
Potamilus alatus (Say, 1817)	1	2	3
Ptychobranchus fasciolaris (Rafinesque, 1820)	6	19	25
Quadrula metanevra (Rafinesque, 1820)	0	0	0
Q. nodulata (Rafinesque, 1820)	0	0	0
Q. pustulosa (Lea, 1831)	0	1	1
Q. quadrula (Rafinesque, 1820)	0	7	7
Tritogonia verrucosa (Rafinesque, 1820)	0	8	8
Totals:	29	259	288

their gills modified as marsupia if normal reproduction were occurring. Histological examination revealed that the male to female ratios for the two target species were not statistically different from 1:1. Both of the target species, as well as other species of unionids, usually maintain a 1:1 male to female ratio (Jirka and Neves, 1992).

Most of the males of the two target species had more than one stage of spermatogenesis present in their gonads, but usually the most advanced stage present dominated the acini of the testes (Table 2). Spermatids were observed in most of the male *Elliptio dilatata* collected, but all the cell types except spermatozoa were also observed. All the cell types except spermatocytes were observed in male *Actinonaias ligamentina*, with sperm morulae and spermatids the most common.

All of the female *Elliptio dilatata* had each of the three egg cell types present in their ovaries (Table 2). The most advanced stage present did not always dominate the alveoli of the ovaries, as was observed in the testes of the males. Most of the *E. dilatata* were in the second stage of oogenesis with oocytes dominating the alveoli. One female was categorized as unknown because the stage of oogenesis could not be determined due to technical difficulties. Hermaphroditism, which has been reported for both of the target species (van der Schalie, 1970; Jirka and Neves, 1992), was observed in one *Actinonaias ligamentina* individual. Most of the acini in this individual contained male gametes, and those with female gametes seemed to be degenerate. Even though the male to female ratios of the target species were not significantly different from 1:1, a majority of the females had empty marsupia (Table 2).

The post-impoundment water temperatures at Farmers, Kentucky, which is located between Moores Ferry and Cave Run Lake, were found to be significantly lower than pre-impoundment temperatures for the months of May through July. There was no significant change in water temperature for August, and a significant increase for September (Table 3). A change in the temperature regime of an aquatic system, especially a decrease, can have drastic effects on freshwater mussels. Water temperature influences the length of the period of development, the time of fertilization (Matteson, 1948), the release of glochidia (Yokley, 1972; Jirka and Neves, 1992), the survival of glochidia before they attach to a host (Tedla and Fernando, 1969), and it is the major environmental factor that regulates the period of glochidial attachment (Matteson, 1948; Zale and Neves, 1982; Neves and Widlak, 1988). Decreases in water temperature can also result in a decreased production of the crystalline style (Allen, 1921), reduce the motility of adult unionids (Yokley, 1972) and influence the growth of adults (Hruška, 1992).

The average monthly discharge at Farmers, Kentucky, increased from pre- to post-impoundment for the months of June through September and decreased during May, but none of these changes were statistically significant (Table 3). Even though these changes were themselves not statistically significant, they were directly related to significant changes in water temperature. The hydrological variability of a river influences the spatial distribution of individuals in a freshwater mussel community (Di Maio and Corkum, 1995), and stream velocity is a major factor

Table 2. Stages of gametogenesis and marsupial contents of *Actinonaias ligamentina* and *Elliptio dilatata* from the Licking River at Moores Ferry, Kentucky. See text for stage and content descriptions.

	Stage of Spermatogenesis					Stage of Oogenesis				Marsupial Contents			
Taxa	1	2	3	4	5	1	2	3	UNK	EE	AE	MG	EM
Actinonaias ligamentina	1	4	0	4	1	-	-	-	-	2	0	3	12
Elliptio dilatata	1	2	3	7	0	2	4	1	1	0	2	1	5
Totals:	2	6	3	11	1	2	4	1	2	2	2	4	17

Table 3. Mean monthly water temperature and discharge for the Licking River at Farmers, Kentucky, during pre- (1966-1979) and post-impoundment (1991-1994) periods.

	Water Temperature (°C)			Discharge (m^3/s)		
Month	1966-79	1991-94	Students-*t*	1966-79	1991-94	Students-*t*
May	16.3	13.4	3.0291*	48.1	27.7	0.8716
June	22.1	17.0	3.4908*	5.6	14.7	-1.2913
July	24.5	21.3	2.0554*	4.0	8.3	-1.4505
August	23.0	22.9	0.1184	7.6	9.0	-0.3339
September	20.7	23.0	-3.1114*	3.4	5.0	-1.0613

*Statistically different at $\alpha = .05$

determining the presence or absence of different species within a freshwater community (Horne and McIntosh, 1979). During periods of high discharge the dilution of sperm and glochidia may occur (Miller and Payne, 1988), and continuous high-turbulence flow can impact adult unionids by eroding the periostracum (Miller *et al.*, 1984).

Even though there were no changes in discharge, and significant decreases in temperature ($p < 0.05$) only during the months of May through July, the impacts of these changes were still biologically important. It is believed that the average monthly discharge and temperature may not be as important as the spikes of discharge, and the corresponding sudden decreases in temperature, that are caused by the releases of hypolimnionic water from Cave Run Lake during important reproductive periods.

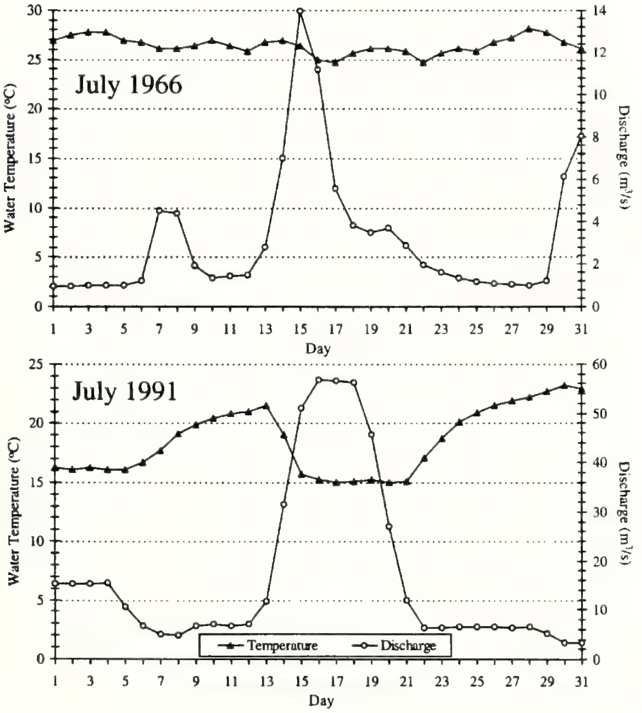

Fig. 2. Licking River at Farmers, Kentucky, mean water temperature (°C) and daily mean discharge (m^3/s) during July 1966 and July 1991.

For example, during July 1966, before the completion of Cave Run Lake, water temperature averaged 26.4 ± 0.85°C and the discharge averaged 3.2 ± 3.2 m^3/s (Fig. 2). During July 1991, after the construction of the lake, the average water temperature was 18.5 ± 2.82°C and the average discharge was 16.8 ± 17.5 m^3/s. There were large spikes of discharge during both of these months. During July 1966 discharge increased and then returned to normal seven days later, resulting in only a 3°C decrease in temperature that did not last long after the increased discharge had subsided. During July 1991 the spike in discharge returned to normal nine days later, and resulted in a 6.5°C decrease in temperature that did not return to its pre-release level until four days later. This single spike of discharge impacted the normal water temperature for a period of 13 days. Sudden, drastic, temperature changes such as these have been reported to cause the abortion of embryos and glochidia (Matteson, 1948; Bruenderman and Neves, 1993).

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Recovery Status of Freshwater Mussels (Bivalvia: Unionidae) in the North Fork Holston River, Virginia

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Abstract: To determine the degree of recovery of mussels from mercury (Hg) contamination in the North Fork Holston River (NFHR) downstream of the Superfund site at Saltville, Virginia (NFHRM 80.3), 19 sites were sampled using catch-per-unit-effort (no./h) sampling method and 3 sites were surveyed with quadrats (0.25 m^2). Nine species of live freshwater mussels were observed in the river, and juveniles were noted at 5 sites (30 juveniles of 4 species). The first mussel assemblage, as defined by numerous animals of multiple species, was located at NFHRM 59.9, approximately 20.4 river miles downstream of Saltville. The greatest number of species was observed at NFHRM 11.0 (5 species), while the greatest mussel density (2.6 mussels/m^2), the greatest number of juveniles (11), and the greatest species richness of juveniles (3 species) were observed at NFHRM 13.5. Random catch-per-unit-effort at surveyed sites, as well as the number of juvenile species observed, were correlated to total Hg, but not methylmercury content, as measured in *Corbicula fluminea* (Müller, 1774) from proximate sites. Based on the appearance of multiple species and age classes, as well as the presence of juvenile mussels, recovery of freshwater mussels begins to occur roughly 20 river miles downstream of the Hg contaminated Superfund site at Saltville.

Key Words: freshwater mussels, Unionidae, North Fork Holston River, mercury, survey

The North Fork Holston River (NFHR) in southwestern Virginia flows in a generally southwestern direction through Bland, Smyth, Washington, and Scott counties to its confluence with the mainstem Holston River in Tennessee (Fig. 1). This Tennessee River tributary is a moderately hard-water stream with $CaCO_3$ concentrations from 35 mg/l to 239 mg/l. Hill *et al.* (1974) described the NFHR as having a high riffle-pool ratio, and a substratum consisting mainly of sand, gravel, and cobble. Average discharge for the period from October 1931 to December 1981 was estimated at 25.7 m^3/s near Gate City, Scott County, Virginia. The $_7Q_{10}$ for this period was estimated at 1.7 m^3/s, and the average monthly summer flow (July through September) is roughly 9.7 m^3/s.

The river is surrounded by moderate agricultural and low urban development, and has historically supported a high faunal diversity. Environmental degradation above Saltville, Virginia has been minimal, but large quantities of elemental mercury (Hg) and chloride salts were discharged into the river at Saltville during this century, contaminating more than 128 km of this river to its confluence with the Holston River in Tennessee (Sheehan *et al.,* 1989; Seivard *et al.,* 1993). Utilizing local salt deposits, the Mathieson Alkali Works began production of soda ash in 1894 at Saltville, Virginia (Seivard *et al.,* 1993). In 1950, the Mathieson Chemical Company (merging with the Olin Corporation in 1954 to become the Olin-Mathieson Chemical Company) began operation and continued until 1972, using Hg in an electrolytic process to convert sodium chloride to chlorine and caustic soda. It is estimated that, in addition to Hg, the plant produced approximately 862 metric tons of $CaCl_2$ and 522 metric tons of NaCl per day for extended periods (Hill *et al.*, 1974). Two settling ponds were created adjacent to the river to store this waste, covering approximately 122 ha. In 1977, the ponds contained an estimated 100 metric tons of Hg (Carter 1977). Seivard *et al.* (1993) estimated that in the final years of plant operation, as much as 1,814 metric tons of salt and 34 kg of Hg were deposited daily into these settling ponds, the surrounding soils, and to the river. An estimated 100 g of Hg per day continued to seep from these ponds into the river (Carter, 1977). The area was designated as a Superfund site by the U. S. Environmental Protection Agency in 1982.

Detrimental effects to the stream fauna were of cat-

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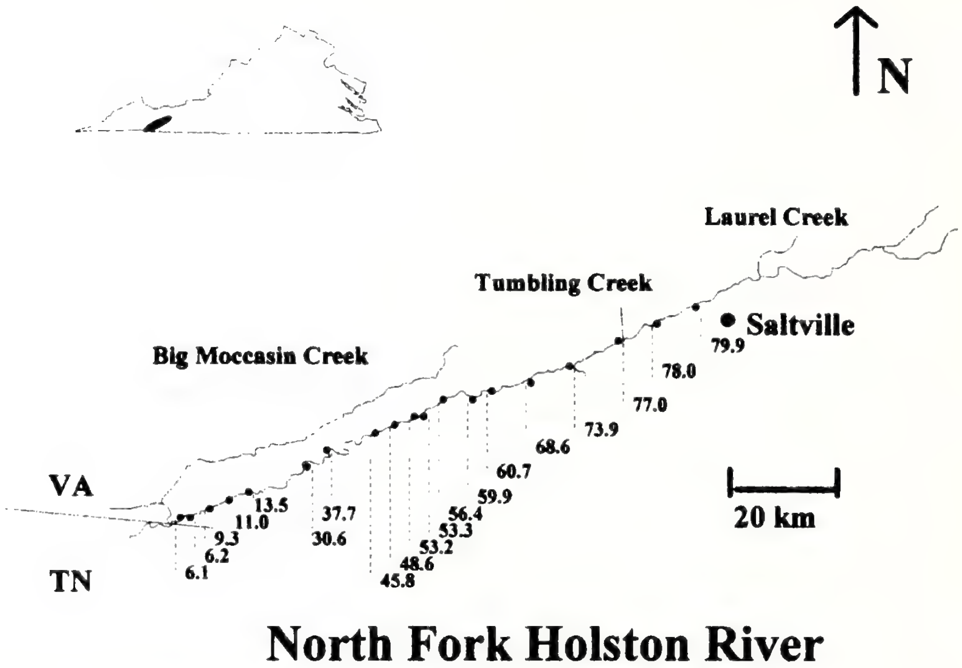

Fig. 1. Locations of survey sites in the North Fork Holston River, Virginia. River miles are measured from the confluence of the NFHR with the mainstem Holston River, Tennessee.

astrophic proportions, essentially extirpating the freshwater mussel fauna downstream of Saltville. The loss of mussels apparently began early in this century. Stansbery (1972) quotes Adams (1915) as stating, "I was told by a resident that occasionally the 'alkali' refuse came down (the NFHR at Saltville) in such quantity as to give the river a milky color." In 1918, Ortmann reported more than 42 species of freshwater mussels above and downstream of Saltville. By 1973, only one species was reported below Saltville, and 11 species upstream (Hill *et al.*, 1974). In 1976, fish tissue obtained at 6 sampling stations along 113 km of river below Saltville contained at least twice the level of Hg allowed by the FDA; therefore, a ban on fish consumption was initiated (Carter, 1977). Mercury contamination continues to be a problem, with concentrations in the tissue of Asian clams (*Corbicula fluminea* Müller, 1774) obtained 121 km downstream of Saltville being eight times higher than levels at an upstream control site (Seivard *et al.*, 1993).

In 1993, a faunal survey noted recovery of benthic invertebrates at North Fork Holston River Mile (NFHRM) 76.7, below the Saltville plant (which is located at NFHRM 80.3). The survey recorded diversity and abundance measures at NFHRM 61.0 that were comparable to an upstream reference site (Woodward-Clyde Engineering 1993). The survey recorded 4 species of mussels at 3 different sites downstream of Saltville: wavyrayed lampmussel, *Lampsilis fasciola* Rafinesque, 1820; pocketbook, *L. ovata* (Say, 1817); mountain creekshell, *Villosa vanuxemensis* (I. Lea, 1838); and rainbow mussel, *V. iris* (I. Lea, 1829). We note that *V. iris* in the NFHR seems to be a complex of 2 sibling species; therefore, every designation of *V. iris* within this document represents this complex.

Other unpublished surveys, as well as translocation records, indicate additional river locations where live mussels occur. A fish survey of the NFHR in 1993 recorded 5 sites containing live mussels (unidentified), from below Saltville to the Tennessee state line at NFHRM 8.0. During this survey, shells of *Lampsilis fasciola, Villosa iris* and flutedshell, *Lasmigona costata* (Rafinesque, 1820) also were collected at 13 sites between river miles 79.6 and 6.2 (Leftwich 1994). Ahlstedt (1979) and Sheehan *et al.* (1989) conducted two mussel translocation programs in the NFHR below Saltville between 1975 and 1985. Seventeen species were translocated to 8 sites. These translocations were conducted with mussels of the Clinch River (CR) at Kyles Ford, Copper Creek in the CR basin, and Big Moccasin Creek, a tributary of the NFHR. Based on these and previous records, there was circumstantial evidence that live mussels were present at 19 sites within the NFHR below Saltville, Virginia.

Our study was conducted to summarize the results of previous unionid surveys of this river, to assess the degree of recovery of freshwater mussels downstream of Saltville, Virginia, and to examine the possible relationship between the distributional pattern of mussels and Hg at selected sites in the river.

METHODS

The degree of recovery of unionid mussels, including location, species richness, abundance, and reproduction in the NFHR downstream of Saltville, Virginia, was determined for 19 sites. The level of survey effort expended at a site was defined by catch-per-unit-effort (CPUE) values recorded, and a predetermined level of CPUE triggered further survey effort. The area of each site was surveyed using a CPUE snorkeling technique (termed random CPUE within this study). Because of differences in ability and experience of snorkelers in locating mussels, the CPUE of the principle investigator (Henley) was used to trigger subsequent sampling. If the CPUE value exceeded 5 mussels/h at a site, transect CPUE surveys were conducted. If the CPUE value exceeded 10 mussels/h, 0.25 m^2 quadrat sampling along existing transect lines also was conducted. All survey work occurred from June to August 1995 during low flow conditions (Table 1 and Fig. 1).

At each site, a random CPUE survey was conducted by a crew of 2 to 5 people to confirm the presence of mussels, their relative abundance, and the position of mussel aggregations. During a random CPUE survey, only visible mussels were counted; few rocks were overturned. Observed mussels were left in position, and their locations were marked with fluorescent flags. As with all survey techniques used, mussels were examined to record species, sex and gravidity, measured for length and width (mm), and returned to the exact location of collection. Random CPUE values were calculated by dividing the number of mussels observed by total effort in hours.

Table 1. Survey sites and associated survey methods conducted on the North Fork Holston River below Saltville, Virginia from June to August 1995.

NFHR Mile	Random CPUE (no./h)	Transect CPUE (no./h)	Transect Quadrats (no./m2)
79.9	X		
78.0	X		
77.0	X		
73.9	X		
68.6	X		
60.7	X		
59.9	X	X	
56.4	X	X	
53.3	X		
53.2	X	X	X
48.6	X		
45.8	X		
37.7	X		
30.6	X	X	X
13.5	X	X	X
11.0	X	X	
9.3	X		
6.2	X		
6.1	X		

We emphasize that transects were not randomly selected, but were positioned to include mussel aggregations discovered during the random CPUE survey. Ten transects per study site were positioned 5 m apart. Catch-per-unit-effort surveys were conducted to include 1 m on either side of transect lines. A 2 m length of rebar with a painted center-line was used during surveys to aid surveyors in remaining within transect width limits. Thus, transect CPUE provided an estimate of relative abundance, and an estimate of observable mussels/m^2. During these surveys, most cobbles larger than 25 cm were overturned (and replaced) to determine the presence of mussels. Survey crews consisted of 3 to 6 individuals of varied experience, but a core crew of the same 3 individuals was always present. Catch-per-unit-effort was calculated as previously described.

For further quantification, 0.25 m^2 quadrats were randomly positioned on existing transect lines using a random numbers table. Each transect was surveyed with 10 quadrats, totaling 100 per study site. Quadrats were excavated to hardpan, or to approximately 25 cm, and substratum was later replaced. Survey crews consisted of 3 to 6 individuals of varied experience, but the core crew of 3 individuals was always present.

The following sample size formula was used to estimate the precision of density estimates with a sample size of 100 quadrats per site (Downing and Downing 1992):

$$n = 1 \cdot \left(\frac{\text{\# mussels estimated per m}^2}{10{,}000 / A} \right)^{-0.5} \cdot D^{-2},$$

where: $A = \text{cm}^2$ covered by each replicate sample (in this case 2500 cm^2),

and $D = SE/m$ = the desired accuracy of density estimates.

Using this formula, a precision of 21% was calculated. All statistical analyses and graphics were conducted and generated using Minitab 10.5[2] (Minitab, Inc., College Station, Pennsylvania). Site quadrat data were analyzed for distributional type using the Chi Squared Goodness-of-Fit Test (Elliott 1977). Because these data fit the negative binomial distribution, a measure of aggregation $(\hat{k})$ was estimated using a maximum likelihood technique (Krebs 1989).

In addition to random and transect CPUE (no./h) and density estimations (no./m^2), data obtained from the combination of these survey techniques facilitated estimations of the number of species present and reproduction within the mussel aggregations at the sites. The presence of juveniles (< 20mm) at a site indicated recent reproduction. Since CPUE (no./h), density (no./m2), and species presence were recorded for sites surveyed, these values were regressed on NFHR mile location. To verify ages of mussels collected, shell material collected from the river was measured for length (mm), and aged by counting external annual rings. These measurements were recorded by species and sex for dimorphic species (Neves and Moyer 1988). From these shell measurements, length and age were regressed for species and sex. Regression equations allowed length measurements of live mussels to be converted to ages.

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[2] Use does not imply endorsement by the U. S. Government.

Relationships between the distributional patterns of bivalves and the presence of Hg in the river were examined. Measures of relative abundance of mussels, number of species present, and methylmercury and total Hg content (mg/l, dry weight) in *Corbicula fluminea* also were regressed on NFHRM (Seivard *et al.*, 1993). Since there was not an exact correspondence between the sites surveyed for Hg content in the Seivard study and the sites surveyed in this study, Hg content values from the nearest sites were used. We collected tissue from *Villosa iris* for determination of total Hg content (mg/l, dry weight) which was analyzed by Hazelton Environmental Services, Inc., Madison, WI using the cold vapor atomic absorption method. For this analysis, 3 replicates of 3 *V. iris* per replicate were taken from NFHRM 84.5, 53.2, and 30.6, totaling 9 mussels sampled per site. For comparison, total Hg content in *C. fluminea* (mg/l, dry weight) collected from the NFHR is also included, as reported by Woodward-Clyde Engineering (1993) and the U. S. Geological Survey (G. Johnson, unpub. data).

RESULTS AND DISCUSSION

A mixture of sand, cobble, boulder, and bedrock substrata characterized survey sites. Ranges of physical measurements recorded at NFHR survey sites were: mean depths of 34.1 cm to 69.7 cm; mean widths of 30.6 m to 59.5 m; and mean velocities of 0.13 m/s to 0.31 m/s. Water temperatures varied from 20 to 32°C.

Nine species of live freshwater mussels were observed in surveys of the NFHR, including the purple wartyback, *Cyclonaias tuberculata* (Rafinesque, 1820); fine-rayed pigtoe, *Fusconaia cuneolus* (I. Lea, 1840); wavyrayed lampmussel, *Lampsilis fasciola*; pocketbook, *L. ovata*; flutedshell, *L. costata*; Tennessee clubshell, *Pleurobema oviforme* (Conrad, 1834); kidneyshell, *Ptychobranchus fasciolaris* (Rafinesque, 1820); rainbow mussel, *V. iris*; and mountain creekshell, *V. vanuxemensis* (Table 2). *L. fasciola* and *V. iris* were observed at all sites surveyed with transect CPUE; *L. ovata* was observed at and downstream of NFHRM 53.2; and, *C. tuberculata, F. cuneolus, L. costata, P. fasciolaris*, and *P. oviforme* were collected only at or downstream of NFHRM 13.5. Measures of relative abundance of mussels varied widely from site to site, including the primary investigator's CPUE and mean transect CPUE. The first mussel assemblage, as defined by numerous animals of multiple species, was located at NFHRM 59.9, approximately 20.4 river miles downstream of Saltville (NFHRM 80.3). Evidence of reproduction (juvenile mussels) was documented at NFHRM 56.4, 53.2, 30.6, 13.5, and 11.0, and included the species *L. fasciola, L. ovata, V. iris*, and *V. vanuxemensis* (Table 3). Relative abundance and evidence of reproduction (juveniles and multiple age classes) was highest at NFHRM 53.2 and 13.5, with decreased levels at other sites.

Table 2. Species observed at 19 sampling sites on the North Fork Holston River, Virginia from June through August 1995. (X, live mussels; *, juveniles.

	North Fork Holston River Survey Site (NFHRM)																		
	79.9	78.0	77.0	73.9	68.6	60.7	59.9	56.4	53.3	53.2	48.6	45.8	37.7	30.6	13.5	11.0	9.3	6.2	6.1
Cyclonaias tuberculata (Rafinesque, 1820)																X			
Fusconaia cuneolus (I. Lea, 1840)																			X
Lampsilis fasciola Rafinesque, 1820	X					X	X	X	X	X	X	X	X	X	X*	X*	X	X	X
Lampsilis ovata (Say, 1817)					X	X			X	X*		X		X	X*	X	X	X	X
Lasmigona costata (Rafinesque, 1820)																		X	
Pleurobema oviforme (Conrad, 1834)															X				
Ptychobranchus fasciolaris (Rafinesque, 1820)																X			
Villosa iris (I. Lea, 1829)				X			X	X*	X	X*	X	X	X	X	X	X	X	X	X
Villosa vanuxemensis (I. Lea, 1838)					X	X	X	X*	X	X	X			X					

Table 3. Survey results from 19 sampling sites on the NFHR from June through August 1995. Transect CPUE, transect CPUE density, and quadrat density values are site means. (CPUE, no./h; density, no./m^2).

	North Fork Holston River Survey Site (NFHRM)																		
	79.9	78.0	77.0	73.9	68.6	60.7	59.9	56.4	53.3	53.2	48.6	45.8	37.7	30.6	13.5	11.0	9.3	6.2	6.1
Investigator CPUE	1	0	0	0	0	0	5	5	5	11	4	3	2	8	22	13	4	3	6
Random CPUE	.50	0.0	0.0	.50	2.0	3.0	11.0	8.0	8.6	34.3	16.5	2.9	4.9	13.6	30.8	17.0	2.3	2.6	5.3
Transect CPUE							2.96	3.75		8.28				5.90	18.6	11.2			*
Transect CPUE Density Estimate							0.04	0.13		0.19				0.15	0.45	0.40			*
Quadrat Density Estimate (± 21%)										2.20					2.60	1.76			*
Juveniles Observed								2		7				5	11	5			
Species with Juveniles Observed								2		2				1	3	2			

*Survey disrupted by the activity of another investigator.

It should be noted that between 1991 and 1995, 852 adult mussels of 9 species were translocated from sites in the Clinch River and upstream of Saltville to NFHRM 13.5 (R. Neves, unpub. data). This was the site with the highest recruitment, as evidenced by the collection of 11 juvenile mussels (Table 3). Collection of juveniles of 3 species indicates that this location is being repopulated by reproduction from translocated animals, because of the prior absence of mussels at this site. The site will presumably become a source of recruitment of juvenile mussels to proximate river reaches in future years.

In general, the number of age classes for all species was highest at NFHRM 53.2, and the number of age classes at downstream sites where random CPUE equaled or exceeded 5 mussels/h remained roughly constant. At most sites, *Lampsilis fasciola, Villosa vanuxemensis, V. iris*, and *L. ovata* were represented by multiple age classes. The number of age classes and relative abundance of *L. fasciola, V. iris*, and *L. ovata* generally increased with distance from Saltville, while age classes and relative abundance of *V. vanuxemensis* decreased. Generally, the number of *V. iris* increased, while the number of *V. vanuxemensis* decreased proceeding downstream. The number of *L. ovata* and *L. fasciola* generally remained similar with downstream river mile, except that the number of *L. fasciola* peaked at NFHRM 53.2.

A comparison of our survey results with those of Ortmann (1918) and Hill *et al.* (1974) revealed that the number of species in the river downstream of Saltville, Virginia has decreased over time (Tables 3 and 4). Between the early 1900s and 1972, the number of reported mussel species decreased from 24 to 1 within the river reach of this study (Ortmann 1918; Hill *et al.*, 1974). From the results of our survey we see that the number of species recorded in the reach has increased from 1 to 9 since 1972 (Table 3). Using maximum ages of live mussels per site, we conclude that recolonization or reproduction within aggregations began at least by the early to mid-1980s. In addition to an increase in the number of species observed downstream of Saltville since the Hill *et al.* (1974) survey, there also appears to have been a shift in the pattern of species richness within the river this century (Ortmann, 1918). Whereas we found species richness to be highest at NFHRM 11.0, the highest species richness in the early 1900s occurred at NFHRM 59.3 (26 species) and 39.2 (24 species) (Table 4). Thus, not only has the number of mussel species reported from the river been drastically reduced, but the spatial distribution of aggregations within the river has shifted.

Descriptive characteristics of mussel assemblages at sites surveyed, such as the total number of mussels observed ($r^2 = 0.40$, $p = 0.18$), the number of juveniles detected ($r^2 = 0.06$, $p = 0.35$), number of age classes for all species ($r^2 = 0.24$, $p = 0.33$), and number of age classes for *L. fasciola* and *V. iris* ($r^2 = 0.23$, $p = 0.33$ and $r^2 = 0.34$, $p = 0.24$, respectively) did not increase significantly with distance downstream of Saltville. Also, random CPUE (no./h) ($r^2 = 0.10$, $p = 0.18$) and mean site quadrat density (no./m^2) ($r^2 = 0.06$, $p = 0.95$) were not correlated to river mile location. Mean transect CPUE was marginally related to NFHRM ($r^2 = 0.64$, $p = 0.06$). The Anderson-Darling test for normality (Sokal and Rohlf 1995) showed that all mussel assemblage characteristics were normally distributed ($p > 0.05$), except random CPUE ($p < 0.001$) and number of juvenile species observed ($p < 0.01$). Transformations indicated by the Box-Cox transformation procedure (Sokal and Rohlf, 1995) did not substantially increase r^2 and associated p values, when these variables were regressed on river mile. Thus, mussel assemblage characteristics did not seem to exhibit a curvilinear relationship to river mile location.

Site quadrat data from surveyed mussel aggregations followed a negative binomial distribution, using the Chi Square Goodness-of-Fit Test (Elliott 1977). Chi square values (x^2), associated p value ranges, and $\hat{k}$ estimates were: NFHRM 53.3, $x^2 = 0.53$ ($0.90 < p < 0.95$) with $\hat{k} = 10.000$; NFHRM 13.5, $x^2 = 6.18$ ($0.25 < p < 0.50$) with $\hat{k} = 0.495$; and NFHRM 11.0, $x^2 = 2.95$ ($0.50 < p < 0.75$) with $\hat{k} =$

Table 4. Species presence at survey sites with comparisons to survey findings of Ortmann (1918) (O) and Hill *et al.* (1974) (H). Holding ponds at Saltville, Virginia are at NFHRM = 80.3. Ortmann site locations are approximate. Ortmann (1918) binomials were revised to conform to current taxonomic names according to Parmalee and Bogan (1998).

	North Fork Holston River Survey Site (NFHRM)																	
	82.8 (O)	79.9	79.0 (H)	59.9	59.3 (O)	56.4	53.2	45.8	45.0 (H)	39.2 (O)	30.6	20.9 (O)	13.5	11.0	8.8 (O)	6.3 (H)	6.2	6.1
Actinonaias ligamentina (Lamark, 1819)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
A. pectorosa (Conrad, 1834)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	-	-
Alasmidonta marginata Say, 1818	X	-	-	-	X	-	-	-	-	X	-	-	-	-	-	-	-	-
A. viridis (Rafinesque, 1820)	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amblema plicata (Say, 1817)	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclonaias tuberculata (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	-	-	X	-	-	-	-
Elliptio dilatata (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	-	-
Epioblasma brevidens (I. Lea, 1831)	-	-	-	-	X	-	-	-	-	-	-	-	-	-	X	-	-	-
E. capsaeformis (I. Lea, 1834)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
E. haysiana (I. Lea, 1834)*	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
E. torulosa gubernaculum (Reeve, 1865)*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
E. triquetra (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
Fusconaia barnesiana (I. Lea, 1838)	X	-	-	-	-	-	-	-	-	X	-	X	-	-	X	-	-	-
F. cor (Conrad, 1834)	-	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	-	-
F. cuneolus (I. Lea, 1840)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	-	-	-	X
F. subrotunda (I. Lea, 1831)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
Lampsilis fasciola Rafinesque, 1820	X	X	-	X	X	X	X	X	-	X	X	X	X	X	X	X	X	X
L. ovata (Say, 1817)	X	-	-	-	X	-	X	X	-	X	X	-	X	X	-	-	X	X
Lasmigona costata (Rafinesque, 1820)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	X	-
Lemiox rimosus (Rafinesque, 1831)	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	-
Lexingtonia dolabelloides (I. Lea, 1840)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	-	-	-	-
Medionidus conradicus (I. Lea, 1834)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	-	-	-	-
Pegias fabula (I. Lea, 1838)	X	-	-	-	-	-	-	-	-	X	-	X	-	-	-	-	-	-
Pleurobema oviforme (Conrad, 1834)	X	-	-	-	X	-	-	-	-	X	-	X	X	-	-	-	-	-
Ptychobranchus fasciolaris (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	-	-	X	-	-	-	-
P. subtentum (Say, 1835)	X	-	-	-	-	-	-	-	-	X	-	X	-	-	X	-	-	-
Quadrula cylindrica (Say, 1817)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
Q. intermedia (Conrad, 1836)	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-
Strophitus undulatus (Say, 1817)	X	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Toxolasma lividus Rafinesque, 1831	X	-	-	-	-	-	-	-	-	-	-	X	-	-	X	-	-	-
Villosa fabalis (I. Lea, 1831)	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
V. iris (I. Lea, 1829)	X	-	-	X	X	X	X	X	-	X	X	X	X	X	-	-	X	X
V. perpurpurea (I. Lea, 1861)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	-	-	-	-
V. vanuxemensis (I. Lea, 1838)	X	-	-	X	X	X	X	-	-	X	X	-	-	-	X	-	-	-
Total Species Observed	16	1	0	3	26	3	4	3	0	24	4	14	4	5	16	1	4	4

*extinct

0.369. Because these data fit the negative binomial distribution, it was appropriate to use $\hat{k}$ as an estimate of site aggregation. It is important to note that higher $\hat{k}$ values reflect lower levels of mussel aggregation or clumping (Elliott 1977). Values of $\hat{k}$ can range from 0 to +∞, with values from 0 to about 8 indicating distributional aggregation (Poole 1974). Thus $\hat{k}$ values > 8 may indicate a distribution of mussels that approaches the Poisson or random distribution. On this basis, mussel distributions at NFHRM 13.5 and 11.0 may be viewed as highly aggregated. Since the $\hat{k}$ value associated with NFHRM 53.2 is > 8, mussels at this site were considered to be approaching a random distribution. Elliott (1977) noted that if the same number and size of quadrats are obtained at survey locations, then $\hat{k}$ may be used to compare aggregations. These requirements were fulfilled in our survey.

Results of our study show no evidence of mussel assemblages immediately below Saltville, between NFHRM 79.9 and 59.9. Between Saltville and river mile 59.9, only isolated individuals of mussel species were observed. Possible explanations for this are the lingering effects of Hg, or insufficient numbers of mussels and suitable fish hosts to support reproduction. A translocation of sufficient specimens of species such as *Villosa iris* and

Lampsilis fasciola into this river reach would allow an assessment of its suitability for recolonization. Comparison of the body burdens of Hg in *Corbicula fluminea* and *V. iris* from the U. S. Geological Survey (G. Johnson, unpub. data), Woodward-Clyde (1993), Seivard *et al.* (1993), and our values shows the need for further Hg monitoring (Table 5 and Fig. 2). Although most studies show Hg levels > 1 mg/l dry weight, content values between studies are inconsistent and likely vary between body burdens in *C. fluminea* versus *V. iris*. Further study is needed to show whether levels of Hg in *C. fluminea* and *V. iris* are comparable for specimens of similar age.

Within this study, random CPUE and total number of species observed were the only mussel assemblage characteristics that were related to total Hg content in *Corbicula fluminea*, as measured by Seivard *et al.* (1993) at proximate survey sites ($r^2 = 0.69, p = 0.02$ and $r^2 = 0.77$, $p = 0.13$, respectively). There was a noticeable absence of younger age classes and a decrease in total mussels observed at sites surveyed at locations upstream of NFHRM 60.0. Total Hg content in *C. fluminea* was inversely correlated to river mile locations ($r^2 = 0.79$, $p = 0.003$), whereas methylmercury content in C. fluminea was not related to river mile ($r^2 = 0.52$, $p = 0.04$), as reported by Seivard *et al.* (1993). Two conclusions seem evident from this information. First, the probability of the effect of Hg on mussel recruitment (recent and historic) in the river increases with proximity to Saltville; and secondly, that effect is seemingly related to total Hg and not methylmercury. It is interesting that measures of relative abundance of mussels estimated by our study were inversely correlated to total Hg content rather than methylmercury content, since methylmercury is known to produce toxic effects in aquatic organisms (Chang *et al.*, 1974; Khan and Weis, 1987; Stinson and Mallatt, 1989; Chen and McNaught, 1992). Perhaps some form of Hg in the total Hg component that was not tested has had a contributing effect.

When examining the relationships between site assemblage characteristics, such as the number of mussel species and random and transect CPUE versus methylmer-

Table 5. Mean total mercury content (mg/l, dry weight) in *Corbicula fluminea* and *Villosa iris* from selected North Fork Holston River sites (G. Johnson, unpub. data; Woodward-Clyde, 1993; Seivard *et al.*, 1993; this study). Settling ponds at Saltville are at NFHRM 80.3.

	Seivard *et al.* (1993) *C. fluminea*		Woodward-Clyde (1993) *C. fluminea*		G. Johnson, unpub. data *C. fluminea*		This Survey *V. iris*	
Site	Mean	s	Mean	s*	Mean	s**	Mean	s
91.5			NP					
88.5			< 0.20					
85.6			< 0.20					
84.5	0.18	0.02					1.54***	1.61
81.3			0.13*					
79.9	3.34	0.42						
76.7			NP					
74.0			0.49*					
71.0			0.52	0.12				
69.9	3.46	0.21			1.50			
66.3			NP					
63.8			< 0.26					
60.7			0.48*					
60.2	2.65	0.04						
58.2			< 0.20					
56.4			< 0.28					
53.2			0.51	0.08			2.67	0.53
51.3	1.79	0.15						
39.7	2.38	0.23						
30.6							5.27***	3.94
30.1	1.94	0.20						
22.8	1.89	0.14						
7.6	1.47	0.13						
4.8					1.40			

*Most standard deviations for Woodward-Clyde data were not included because some data were presented as < 0.20 or of only one datum; **standard deviations of G. Johnson (unpub. data) not provided; ***one datum was comparatively high for the site (3.39 mg/l, dry weight); ****one datum was comparatively high for the site (9.73 mg/l, dry weight).

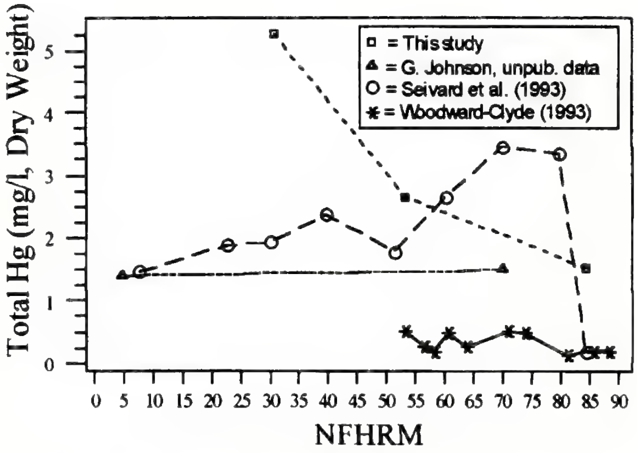

Fig. 2. Mean total mercury content (mg/l, dry weight) in *Corbicula fluminea* and *Villosa iris* from selected sites on the North Fork Holston River (G. Johnson, unpub. data; Woodward-Clyde, 1993; Seivard *et al.*, 1993; this study). Accuracy of data points of Woodward-Clyde (1993) are suspect (represented at < 0.20 or by one datum). Settling ponds at Saltville are at NFHRM 80.3.

cury content in *Corbicula fluminea* (Seivard *et al.*, 1993), an interesting phenomenon is seen. At NFHRM 45.8 through 30.6, decreases in site assemblage characteristics occur, and associated with these decreases are increases in total Hg in *C. fluminea* at NFHRM 39.7(Tables 3 and 5 and Fig. 2). Although total Hg is seemingly related to reduced mussel assemblages in this reach of the river, further survey would be required to confirm this apparent effect. Seivard *et al.* (1993) found a general decreasing trend in total Hg content in *C. fluminea* downstream of Saltville, Virginia. The exception to this decreasing trend was observed at NFHRM site 39.7, where total Hg content increased (Seivard *et al.*, 1993). Mercury content in *C. fluminea* measured by the U. S. Geological Survey (G. Johnson, unpub. data) also decreased with distance from Saltville, whereas similar data measured by Woodward-Clyde (1993) showed no trend (Table 5 and Fig. 2). Additional testing is needed to determine whether the decrease in total Hg with downstream distance from Saltville is real or spurious. If the elevated Hg levels in *C. fluminea* near NFHRM 30.0 through 40.0 are representative of elevated levels of Hg in river sediment, then mussel aggregations downstream, such as at NFHRM 13.5 and 11.0, could be at future risk if such a contaminated sediment load is being transported downstream.

When considering the re-establishment of mussel species reported by Ortmann (1918) in the river downstream of Saltville, Virginia, and the current absence of most of those species in the upper and lower river, the need for a mussel restoration program becomes apparent. Of the 33 species reported by Ortmann (1918) downstream of Saltville, Hill *et al.* (1974) and Barr *et al.* (1993) observed 11 and 10 of these species upstream, respectively. Consequently, translocation of mussels from another river within the same basin will be essential for the re-establishment of mussel species that were present historically.

In addition to previous translocation, habitat requirements of translocated species, and possible contaminant effects, the selection of sites should be guided by evidence of current reproduction, historical pattern of mussel aggregations, and the availability of suitable fish hosts. Our results indicate that NFHRM 11.0, 13.5, 30.6, and 53.2 are suitable sites for reintroduction. The river reach between NFHRM 40 through 60 should be evaluated in further assessments of site suitability, as this stretch of the river historically supported a high diversity of species (Ortmann, 1918).

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Historical and ontogenetic changes in shell width and shape of land snails on the island of Kikai

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Abstract: Patterns of change in shell width over a period of 35,000 years are documented in three fossil land snail species, *Euhadra pachya* (Pilsbry, *Phaeohelix phaeogramma* (Ancey), and *Coniglobus mercatorius daemonorus* (Pilsbry) on the island of Kikai in the Ryu-kyu Islands of the southwest part of Japan. The shell width of these species fluctuated through time, and the patterns were mostly synchronized among the three species. The temporal variations in shell width among fossil populations were far larger than geographical variations in shell width among modern populations. Since Kikai Island has been isolated from the other islands in the region, these morphological changes are regarded as genuine changes that have occurred within the island. The temporal changes in width were accompanied by distinct changes in spire indexj (height/width) within a single species. The patterns of change in width are correlated with the pattern of change in the climate since 35 Ka (35,000 years ago).

Key Words: body size, fossil, Kikai Island, land snail, shell morphology

A number of studies on the patterns and mechanisms of phenotypic evolution have been undertaken on fossil land snails in island systems (*e. g.*, Gould, 1969, 1984, 1989; Goodfriend and Gould, 1996; Chiba, 1996). Important contributions have been made to the study of patterns of Quaternary biogeographic change in island snails within an island (Goodfriend, 1987, 1993; Goodfriend and Mitterer, 1993; Goodfriend *et al.*, 1994; Cook *et al.*, 1993). In the present study, the morphologies of fossil and modern land snails from Kikai Island in the southwest part of Japan were studied, because it offers an excellent example to see morphological changes of lineages within an island.

Kikai Island, in the northern part of Ryukyu Islands, is a small island, approximately 60 km^2 in area and lower than 200 m above sea level. It has been isolated from other islands in the region since 100,000 years ago. The island has eolianite dunes that were produced during the late Pleistocene and Holocene no more than 40,000 years ago. Stratigraphic studies and ^{14}C dating of the eolianite dunes on Kikai have been performed by Mitsui and Kigoshi (1966), Nakagawa (1967), Kakuta (1977), and Naruse and Inoue (1987), and these studies have shown that paleosoil layers have been produced four times: 32 - 31 Ka (thousand years ago) (period II), 29 - 27 Ka (period III), 22 Ka (period IV) and 3 - 2.5 Ka (period V). Fossil land snails have been reported from these paleosoil layers. In addition, land snail shells are found in a calcareous sand layer just above the base deposits of the dune. This layer consists of coarse sand, blocks of beach rocks and marine shell fragments, and includes fossils of the land snails. The age of the snails from this calcareous sand layer is 35,600 yr (Naruse and Inoue, 1987), and the layer is designated period I. Fossil land snails are found in the dune deposits, and three species of pulmonates, *Euhadra pachya* (Pilsbry, 1902), *Phaeohelix phaeogramma* (Ancey, 1888), and *Coniglobus mercatorius daemonorus* (Pilsbry, 1901), are the most common species. *E. pachya* is endemic to Kikai, and *C. mercatorius daemonorus* is an endemic subspecies of Kikai Island.

Although it is not apparent how these species divide resources to avoid competition, *Phaeohelix phaeogramma* and *Coniglobus mercatorius daemonorus* seem to have similar niches: both live sympatrically under leaf litter or rocks on the ground and never climb trees. *Euhadra pachya* became extinct during the Holocene and so its habitat is not known. On the basis of observations of *E. herklotsi*, the most closely related species to *E. pachya*, it is assumed that the niche of this species is similar to that of *P. phaeogramma* and *C. mercatorius daemonorus*.

Here, we show evidence of distinct changes in shell width that have occurred in the three species during the past 35,000 years. The morphological patterns are synchronized among the three species. Distinct changes in shell shape follow the increase or decrease in shell width. Variations in shell shape may have close relationships with ontogenetic changes in shell shape (Foote and Cowie, 1988; Gould, 1984). We show that patterns of historical changes in shell shape are associated with ontogenetic changes in shell shape. In addition, the correlates of climatic changes and other factors on the patterns of morphological changes are discussed.

MATERIALS AND METHODS

Twenty-two samples of fossil and living snails were collected from 18 localities (Fig. 1 and Table 1). Specimens used for the analysis were all adult snails that had a reflected, thickened shell lip. Specimens in each modern sample were taken from an area of 25 m^2. The vegetation at each modern site was categorized as shrub or forest (Table 1). Sites categorized as shrub were sites with plants lower than 1 m in height, and sites categorized as forest were sites with plants higher than 2 m in height. The leaf litter of the former was more than 30 cm in depth, but that of the latter was very thin. Forest and shrub did not coexist in a sampling site, because the area of each sampling site was small. All of the samples used in this study were deposited in University Museum, Shizuoka University (SUM).

Data of ^{14}C ages previously reported for each layer are adopted for the age of the samples used in the present study (Table 1). These data were obtained by dating shells. In addition, the ages of seven fossil samples were examined by ^{14}C dating. The ^{14}C ages were determined by standard methods with benzene-liquid scintillation (Stipp *et al.*. 1974; Gupta, 1985).

All fossil samples were collected from paleosoil layers except for one sample (P16L) that was from the calcareous sand layer. *Phaeohelix phaeogramma* and *Euhadra pachya* were found in all fossil samples, but *Coniglobus mercatorius daemonorus* was not found in sample P16L. *E. pachya* was not found in the modern samples. In total, 951 specimens were measured. Characters measured were shell height (H), width (W) and number of whorls. An edge of the protoconch was defined zero whorls. Shell shape was represented by spire index (H/W) (Cain, 1977).

All three species examined here have determinate growth. Shell width and height at each whorl number (Wn and Hn in Fig. 2) were measured on the shell which was cut along the coiling axis. Ten snails randomly selected from the set of modern and fossil shells were cut in order to measure ontogenetic change for each species. Spire index at each whorl number was obtained by Hn/Wn, and ontogenetical changes of these characters were examined. In addition, relationships between shell width, number of whorls and spire index were examined in adult snails to estimate

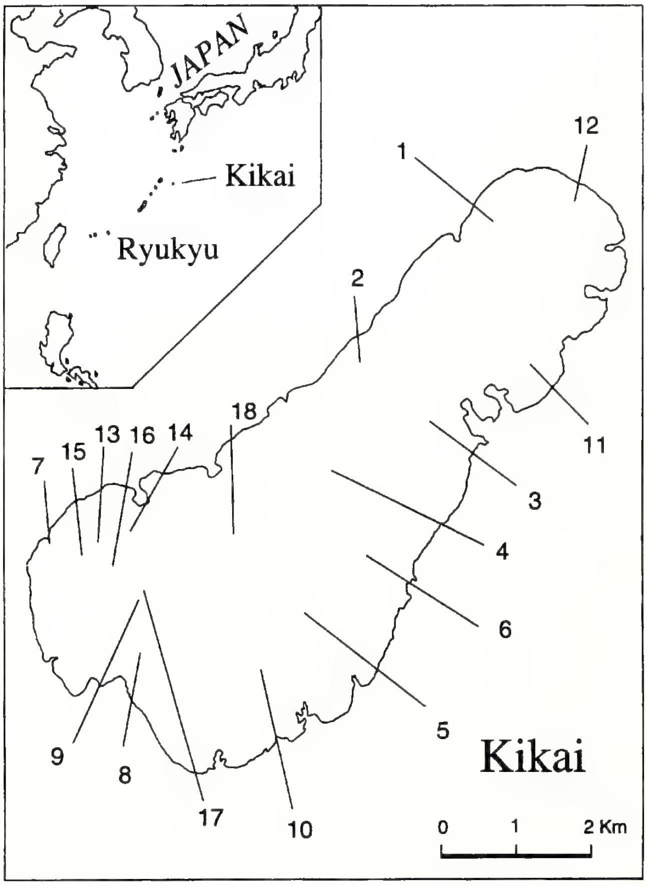

Fig. 1. Map of the island of Kikai showing the numbered sample locations.

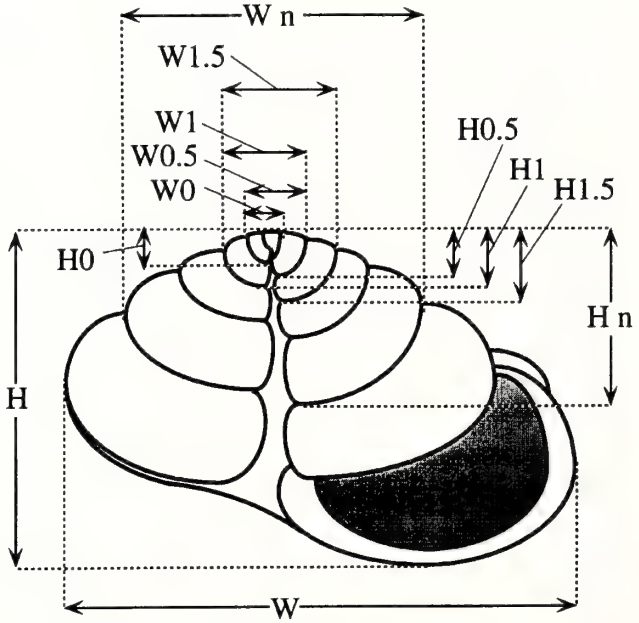

Fig. 2. Description of shell measurements on a cross section of a shell. W: width of an adult shell, H: height of an adult shell, W0: width of a protoconch, Wn: width of the nth whorl (n=0.5, 1, 1.5, 2...), H0: height of protoconch, Hn: height of the nth whorl (n=0.5, 1, 1.5, 2...).

Table 1. Modern and fossil samples of *Phaeohelix phaeogramma* (P.p.), *Coniglobus mercatorius daemonorus* (C.m.), and *Euhadra pachya* (E.p.) from Kikai (H=Holocene, P=Pleistocene, M=modern, U=upper section, M=middle section, L=lower section). Accession number of University Museum, Shizuoka University (SUMFC) for each sample is also presented. Asterisked ages are from Naruse & Inoue (1987). Vegetation and height above sea level for each sample site are also presented.

				Number of specimens				Height above	Accession
Loc.	Sample	Period	Age	P. p.	C. m.	E. p.	Vegetation	sea level (m)	number
1	M1	Modern		22	13	0	forest	15	SUMFCC0051
2	M2	Modern		20	24	0	shrub	25	SUMFCC0052
3	M3	Modern		24	20	0	forest	8	SUMFCC0053
4	M4	Modern		20	30	0	forest	103	SUMFCC0054
5	M5	Modern		20	20	0	forest	150	SUMFCC0055
6	M6	Modern		20	12	0	forest	198	SUMFCC0056
7	M7	Modern		20	17	0	shrub	5	SUMFCC0057
8	M8	Modern		18	12	0	shrub	43	SUMFCC0058
9	M9	Modern		20	14	0	shrub	50	SUMFCC0059
9	P9	III	26800±500	46	16	28	unknown	50	SUMFCC0060
10	M10	Modern		25	21	0	shrub	55	SUMFCC0061
11	M11	Modern		21	10	0	forest	7	SUMFCC0062
12	H12	V	670±40	21	10	4	unknown	4	SUMFCC0063
13	H13	V	3030±90	24	5	6	unknown	15	SUMFCC0064
14	H14	V	3200±60*	36	12	15	unknown	14	SUMFCC0065
15	P15U	IV	22200±300	24	10	11	unknown	20	SUMFCC0066
15	P15L	II	31300±700	26	8	8	unknown	14	SUMFCC0067
16	P16U	III	27900±630*	10	10	8	unknown	32	SUMFCC0068
16	P16M	II	32600±870*	26	16	17	unknown	30	SUMFCC0069
16	P16L	I	35600±1500*	16	0	5	unknown	29	SUMFCC0070
17	P17	III	29100±400	45	18	22	unknown	46	SUMFCC0071
18	P18	II	31700±850	13	6	6	unknown	32	SUMFCC0072

effects of determinate growth of these species on their adult shell morphologies.

Geographical variation in shell width of the modern populations was examined to estimate the relationship between geographical variation in shell width and variation of local environmental conditions. Differences of shell width among modern samples were analyzed statistically with ANOVA. Temporal changes of shell width and spire index were examined by placing the populations in their stratigraphic context.

RESULTS

The ^{14}C age data presented in this study were consistent with the previous ^{14}C age data for the dune deposits of Kikai Island (Kakuta, 1977; Naruse and Inoue, 1987). ^{14}C age anomalies in snail shells were reported by Goodfriend and Stipp (1983) and Goodfriend (1987). Age anomalies in samples of living *Phaeohelix phaeogramma* and *Coniglobus mercatorius daemonorus* from Kikai have been examined and ranged from 300 to 800 years (Takahashi and Wada, 1998). This imples that the samples are 300 ~ 800 years younger than the ^{14}C ages, and that the order of ages of two samples in which the interval of ages is less than 1000 years is not clear. However, the affect of these age anomalies on the overall patterns of morphological changes are minor, because the intervals between most of the fossil samples examined in this study are greater than 1000 years except for those between the three Pleistocene samples (P15L, P16M, P18) and between the Holocene samples.

Shell width of the three species showed similar changes over the past 35,000 years (Fig. 3). Shells reached a maximum width at approximately 28 Ka, and stayed wide until 22 Ka. Specimens in samples H12 - H14 (2.6 - 3.2 Ka) were far smaller than the specimens of the sample P15U (22 Ka), indicating that a reduction in shell width had occurred between 22 and 3.2 Ka. The shell width of the modern samples was similar to that of samples from the Holocene deposits (2.6 - 3.2 Ka) and that of the samples during 29 - 35 Ka.

Because of the historical changes in shell width, the mean widths in some samples of *Phaeohelix phaeogramma* (28 ~ 22 Ka) were greater than the mean width of modern *Coniglobus mercatorius daemonorus*, which was approximately 1.2 times greater than the mean width of modern *P. phaeogramma*. In spite of the changes in width, however, the ratios of widths among *C. mercatorius daemonorus*, *P. phaeogramma* and *Euhadra pachya* within the same sample remained mostly constant. This constancy in ratios of width

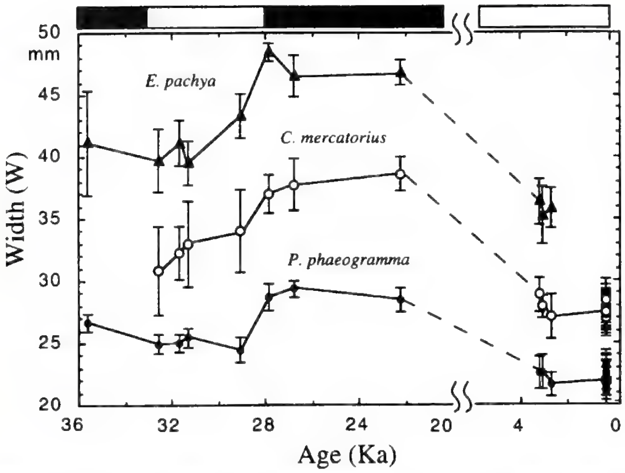

Fig. 3. Patterns of temporal change of sample means of shell width for three species through 35,000 years. Closed circles: *Phaeohelix phaeogramma*, open circles: *Coniglobus mercatorius daemonorus*, closed triangles: *Euhadra pachya*. Each bar indicates one standard deviation. The closed bar at top indicates periods of wetter climates than other periods.

resulted from simultaneous changes in width in the same direction in all three species.

The spire indices of *Coniglobus mercatorius daemonorus* and *Phaeohelix phaeogramma* also showed a pattern of change that was similar to that of shell width (Fig. 4). Spire index of the shells of *C. mercatorius daemonorus* increased at 28 Ka, and then it became low at 3.5 Ka. Spire index of *P. phaeogramma* also became low at 3.5 Ka. Especially, the spire index of modern *C. mercatorius daemonorus* is distinctly different from that of 28 - 22 Ka (Fig. 4). Within this species, the spire index of the modern samples and those of the fossil samples before 28 Ka were closer to each other than the samples of other ages (Fig. 4). *Euhadra pachya* also showed temporal changes in spire index, but the specimens in the fossil samples from 28 Ka to 22 Ka had a lower spire index than the samples of other ages (Fig. 4).

Ontogenetic changes of spire index showed that the spire index (H0/W0, H0.5/W0.5, H1/W1 ... H/W) of *Coniglobus mercatorius daemonorus* and *Phaeohelix phaeogramma* decreased from juvenile to middle stage (whorl numbers fewer than 3), but increased after the middle stage (Fig. 5). However, the spire index of *Euhadra pachya* monotonically decreased during ontogeny (Fig. 5). All of the specimens of a species showed consistent ontogenetic patterns. A similar pattern is seen in spire index versus width.

There were nearly perfect correlations among width, number of whorls and spire index of adult shells (Fig. 6). There was a positive correlation between shell

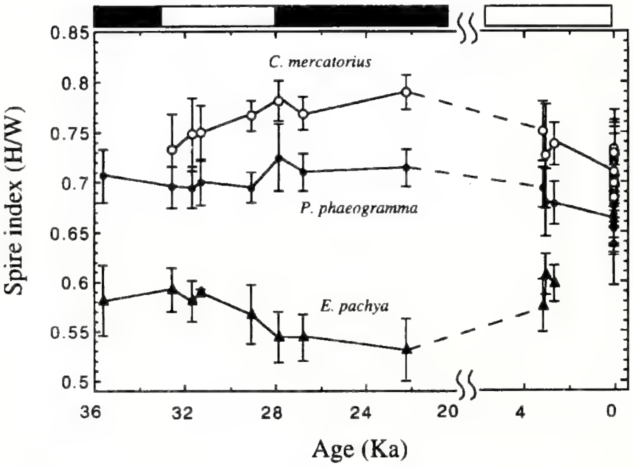

Fig. 4. Patterns of temporal change of sample means of spire index for three species through 35,000 years. Closed circles: *Phaeohelix phaeogramma*, open circles: *Coniglobus mercatorius daemonorus*, closed triangles: *Euhadra pachya*. Each bar indicates one standard deviation. The closed bar at top indicates periods of wetter climates than other periods.

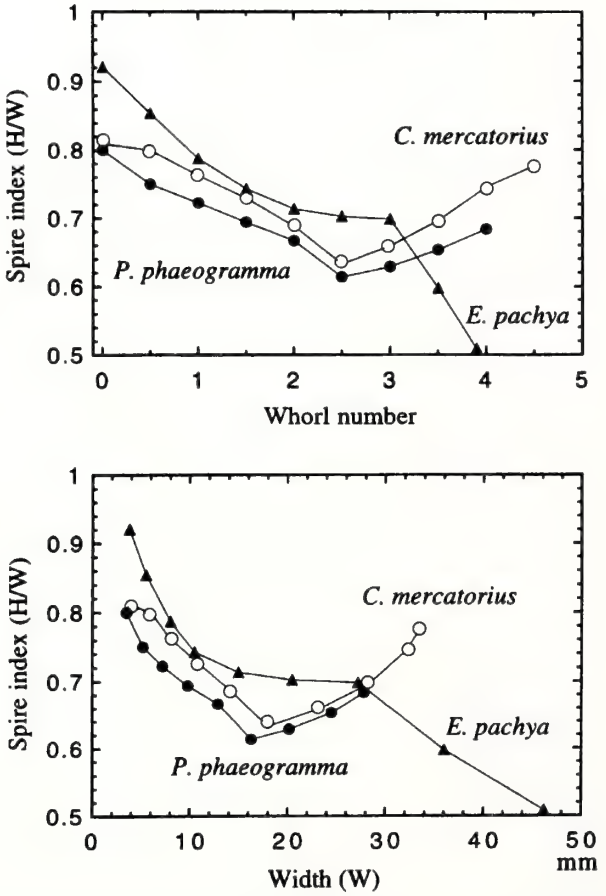

Fig. 5. Ontogenetic changes of spire index versus whorl number and shell width for representative specimens of the three species. Closed circles: *Phaeohelix phaeogramma*, open circles: *Coniglobus mercatorius daemonorus*, closed triangles: *Euhadra pachya*.

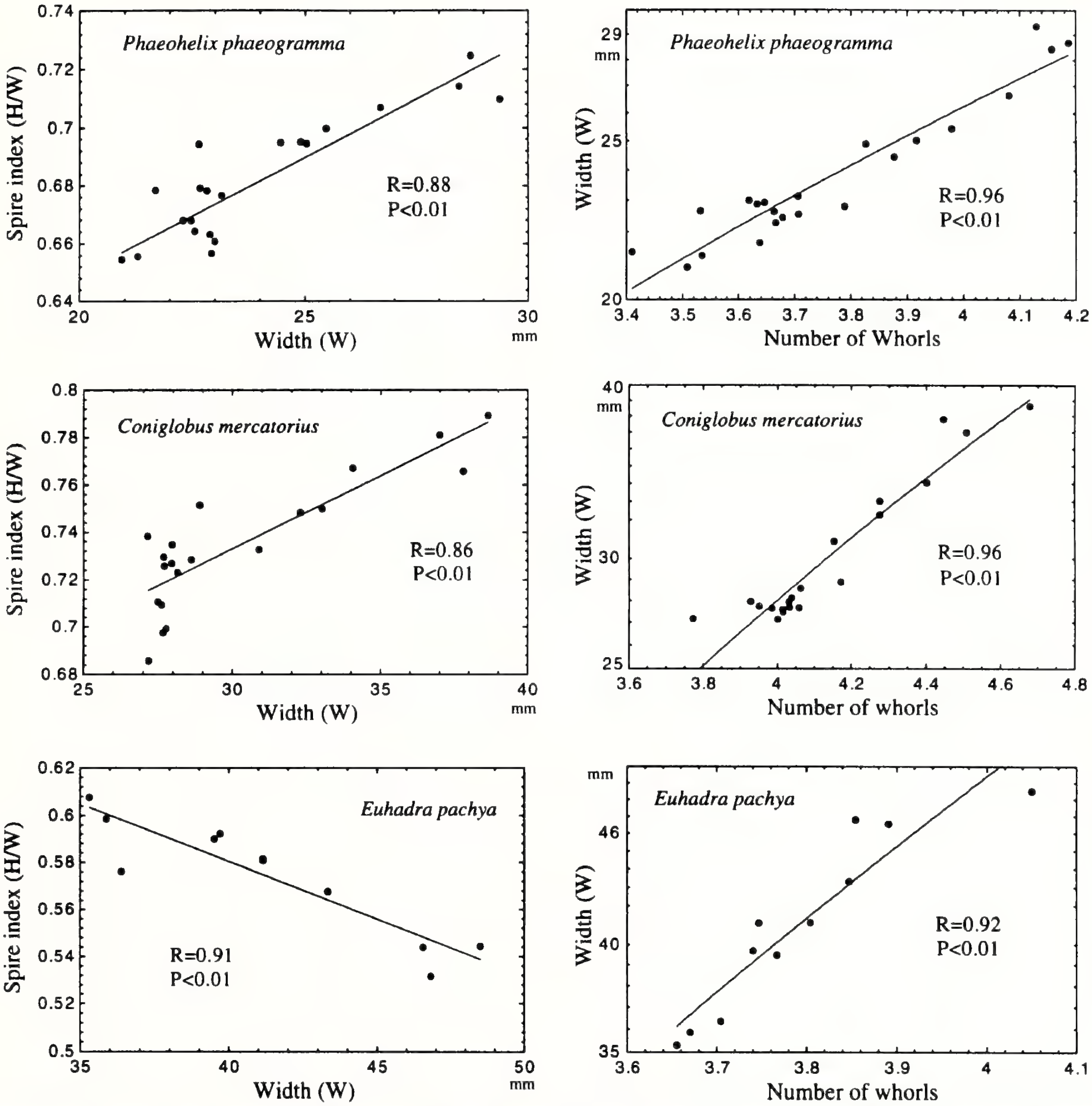

Fig. 6. Relationships among sample means of adult shell width (W), spire index (H/W), and number of whorls. A positive correlation between the number of whorls and the width is found in all species. A positive correlation between width and spire index is found in *Phaeohelix phaeogramma* and *Coniglobus mercatorius daemonorus*, but that of *Euhadra pachya* shows a negative correlation. Correlation coefficients (R) and *P* values are shown.

width and the number of whorls in the three species. *Coniglobus mercatorius daemonorus* and *Phaeohelix phaeogramma* showed a positive correlation between width and spire index. *Euhadra pachya* had a negative correlation between width and spire index. Correlation coefficients for these relationships (see Fig. 6) were all statistically significant ($P<0.01$ in all cases, see Fig. 6).

Geographical variations in shell width observed in the modern samples of *Phaeohelix phaeogramma* are shown in Fig. 7. Shell widths of samples M8, M9, and M10 collected from shrub were significantly smaller than all of the samples collected from forest ($P<0.05$). Shell width of sample M2 from shrub did not differ significantly ($P>0.05$) from any forest samples except sample M6 ($P<0.05$), and sample M7 from shrub did not differ significantly from any of the forest samples ($P>0.05$). As a whole, however, shell widths of snails from forest tend to be larger than those from shrub. Two modern samples of *Coniglobus mercatorius daemonorus* (M9 and M10) from shrub had statistically smaller shell widths than samples from forest ($P<0.05$),

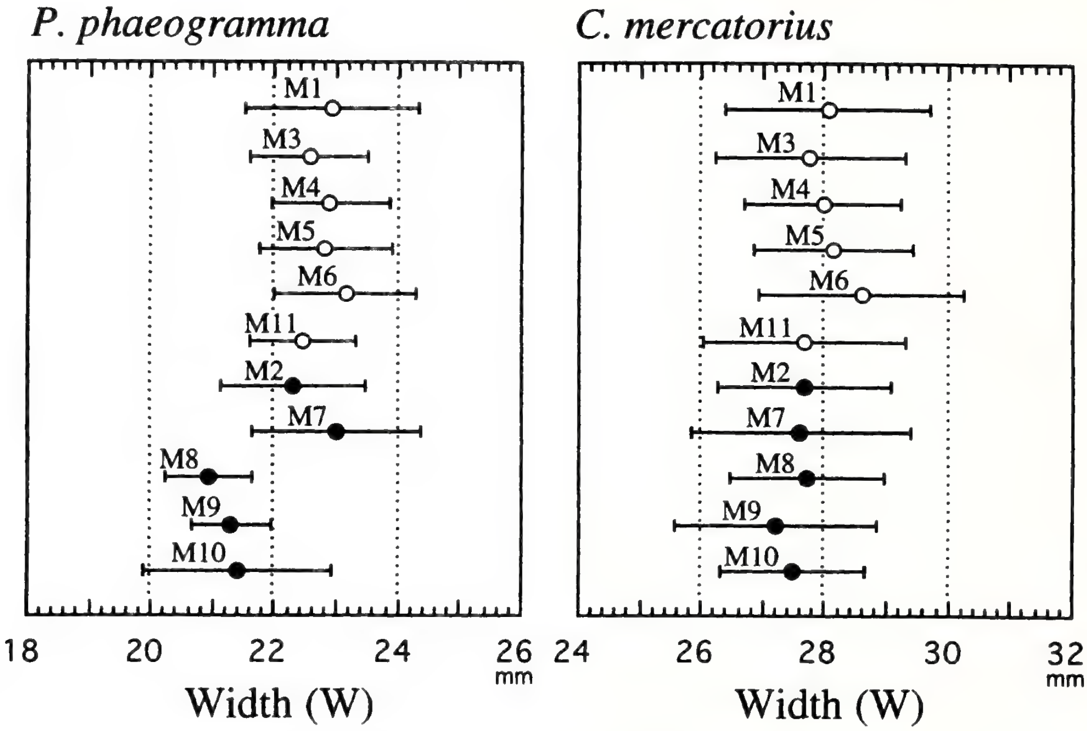

Fig. 7. Geographical variation in sample means of shell width among modern samples of *Phaeohelix phaeogramma* and *Coniglobus mercatorius daemonorus*. Open circles: samples collected from forest, closed circles: samples collected from shrub. Each bar indicates one standard deviation.

but shell width of other samples from shrub did not differ significantly from forest samples ($P>0.05$).

A significant association of shell width and the height above sea level of the modern sampling site (correlation coefficient $R=0.70$, $P<0.05$) was found in *Coniglobus mercatorius daemonorus* (Fig. 8). However, the scatter is wide and the significance is probably dependent on only a few of the outlying points. In addition, association of these was not clear in *Phaeohelix phaeogramma* ($R=0.28$, $P>0.05$).

DISCUSSION

The synchronized patterns of change in shell size and shape of *Euhadra pachya* and *Coniglobus mercatorius daemonorus* are regarded as genuine changes that have occurred within the island and not a reflection of migration within the island or from elsewhere, because these species are endemic species and subspecies respectively, and because the morphological variation among the modern populations was far smaller than that among samples with different ages. Because Kikai Island has been isolated from other islands since the last ice age (Nakagawa, 1969), the pattern of change documented in *Phaeohelix phaeogramm* also does not reflect immigration from other areas.

The morphological patterns showed that shell width of *Phaeohelix phaeogramma* seemed to decrease during 36 - 32 Ka. Shell widths of the three species remained low during 32 - 29 Ka, and increased after 29 Ka, and reached maximum during 28 - 22 Ka. This latter period corresponds to the time when sea level was lowest. Although there is no fossil record for the period 22 - 4 Ka, reduction of shell width occurred during this period when sea level rose. Yasuda (1987) has suggested that the Japanese islands were wetter 50 - 33 Ka and 28 - 25 Ka than other periods. Although climate of the main parts of the Japanse islands became slightly dry after 25 Ka, the relative increase of the land height of Kikai (approximately 100 m) due to the fall of sea level during 28 - 20 Ka may be responsible for the wettest climate in the island during this period. The periods with wet environments (50 - 33 Ka and 28 - 20 Ka) roughly correspond to the periods when shell width was larger (periods older than 32 Ka and 28 - 22 Ka). Thus, increased shell width appears to be correlated with wet conditions.

There are many environmental factors that can affect shell size, *e. g.*, moisture, temperature, density, predation, and random effects, but temperature and moisture may be the most important factors (Goodfriend, 1986; Emberton, 1994). Moisture level is the best documented environmental correlate of shell size, and a positive correlation between moisture and size or aperture area has been

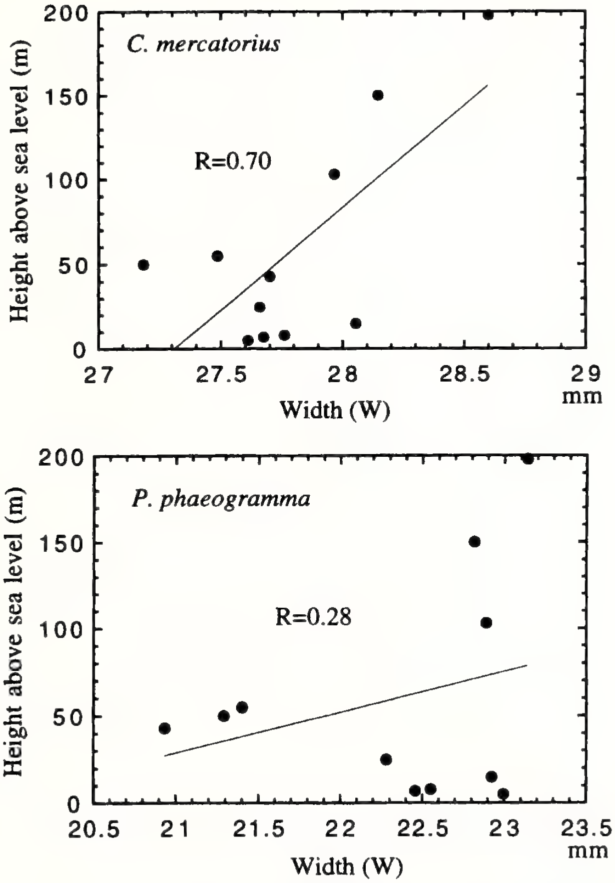

Fig. 8. Relationship between mean shell width of a sample and the height above sea level for samples of modern *Phaeohelix phaeogramma* and *Coniglobus mercatorius daemonorus*.

reported by many authors (*e. g.*, Rensch, 1932; Heller, 1975, 1979; Goodfriend, 1986; Emberton, 1994). In this study, larger shell widths of living snails from forest than from shrub suggest a similar relationship with moisture. The correlation of the morphological pattern with the supposed pattern of climatic change suggests that the historical change in shell width may have been induced by the climatic change.

There are several possible processes by which environmental changes could induce changes in shell width. These changes may be ecophenotypic responses to the changing environment. Distinct non-genetic changes in shell morphology can be created by environmental changes without genetic changes (Gould, 1984). However, these changes may be genetic and evolutionary changes. Breeding experiments on a number of land snail species have shown that size differences among populations have a high heritability (Cook, 1967; Murray and Clarke, 1968; Cook and Cain, 1980; Johnson *et al.*, 1993). Cain (1977) suggested that natural selection for differences in feeding behavior have resulted in divergence of shell height (and see Cook and Jaffar, 1984; Heller, 1987; Emberton, 1994). It is difficult to determine whether the observed changes are genetic or non-genetic. Regardless of the basis of the changes, however, the relationship of climate and shell morphology in the history of these land snail species is affirmed.

There are at least two possible causes for the patterns of historical changes in spire index. First, these changes may reflect adaptation for environmental conditions. Shells with high spires are adaptive in environments with high humidity, because high moisture conditions permit more diverse foraging activities and should mechanically favor higher spires (Cain, 1977; Emberton, 1994). However, in the present examination, shells with a high spire index were not necessarily found in the fossil samples of the period with wet environments. For examples, shells of *Euhadra pachya* became flatter during the period with wetter environments.

Second, these changes may reflect ontogenetical relationships among characters. There was a nearly perfect correlation between shell width and number of whorls, and this relationship implies that a larger shell is a shell with more whorls. Climate affects the number of whorls or shell width at which growth ceases, thereby affecting spire index. A species that increases its spire index with increasing number of whorls and shell width after the middle stage produces an adult shell with low spire index by becoming adult at a reduced number of whorls, thereby decreasing shell width. Therefore, patterns of change in spire index can be interpreted as by-products of the change in numbers of whorls and shell width because of the correlation between width and spire index that is determined ontogenetically. A positive or negative correlation between spire index and shell width observed in the samples of adult snails reflects this ontogenetical relationship between characters.

A positive correlation between spire index and shell width in *Phaeohelix phaeogramma* and *Coniglobus mercatorius daemonorus* and a negative correlation between these parameters in *Euhadra pachya* imply that the high spire index of *P. phaeogramma* and *C. mercatorius daemonorus* and low spire index of *E. pachya* of 28 ~ 22 Ka were created by increasing the shell width by increasing the number of whorls (Fig. 6). The temporally fluctuating patterns of shell shape presented in this study may be induced by fluctuations of shell width that are induced by climatic change.

A correlation between spire index and shell width is found in most land snails (*e. g.*, Goodfriend, 1986). It has been claimed that a non-adaptive change of morphology can occur when the characters are closely linked to size (Gould, 1966, 1969, 1971, 1984). For example, heterochrony, an important cause of evolutionary novelty (Gould, 1977; McKinney, 1986), could produce shape variations as a result of a change in size or a change in the time to maturation. The present study suggests that temporal change in shell form can occur in association with a change in shell width. This idea implies that changes in shell form of the three species could simply be a direct consequence of a change in number of whorls, and may not be adaptive. Although it is difficult to demonstrate that they are non-adaptive at present, the presence of opposite trends in species with similar life styles suggests that the occurrence of non-adaptive change in shell shape can not be ruled out.

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New acavid land snails from Madagascar

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Abstract: Descriptions are given of *Ampelita akoratsara* sp. nov., *A. ambanianae* sp. nov., *A. analamerae* sp. nov., *A. anjanaharibei* sp. nov., *A. ivohibei* sp. nov., *A. josephinae* sp. nov., *A. masoalae* sp. nov., *A. ranomafanae* sp. nov., *A. raxworthyi* sp. nov., *A. (Eurystyla) griffithsi* sp. nov., *Clavator griffiths-jonesi* sp. nov., *C. masoalae* sp. nov., and *Helicophanta gargantua* sp. nov. Discovery of *A. (Eurystyla) griffithsi* sp. nov. greatly expands the geographical, ecological, and morphological ranges of its subgenus. Madagascar's 115 species of acavids are in drastic need of revision.

Key words: Gastropoda, Stylommatophora, Acavacea, *Ampelita, Clavator, Helicophanta*

This paper is the fifth in a series on the Acavidae of Madagascar (Emberton, 1990, 1994, 1995a, 1995b). A recent survey and inventory of Madagascar has yielded extensive collections of acavids; identifications are incomplete but so far have yielded the thirteen new species described herein. With these additions, Madagascar's recognized species of acavids (Fischer-Piette *et al.*, 1994) now total 115.

METHODS AND MATERIALS

Materials were collected 1992-1995. Identifications and comparisons were made using Fischer-Piette *et al.* (1994) and available collections. Measurements were made using vernier calipers. Due to ongoing habitat destruction and the urgency of making this fauna known to conservationists and systematists, only conchological descriptions have been given, except for one case in which dissection of the genitalia was required for generic assignment.

SYSTEMATICS

Higher classification follows Nordsieck (1986). Latitudes and longitudes are given in degrees and minutes. Types are placed in the United States National Museum, Washington, D.C. (USNM); the Australian Museum, Sydney (AMS); the Muséum national d'Histoire naturelle, Paris (MNHN, which does not assign catalog numbers to types); and the Academy of Natural Sciences of Philadelphia (ANSP). Prior, working catalog numbers of the Molluscan Biodiversity Institute (MBI), are also given, as they provide access to an ecological database of stations, available on request. MBI catalog numbers consist of station number, reference number of the species within the station, D (dry) or A (alcohol-preserved), and H (holotype) or P (paratype) or PR (paratype "representative" that is illustrated and/or described). In lot descriptions, ad means adult(s), juv juvenile(s).

Class GASTROPODA
Subclass PULMONATA
Order STYLOMMATOPHORA
Suborder SIGMURETHRA
Infraorder ACHATINIDA
Superfamily ACAVOIDEA
Family ACAVIDAE Pilsbry, 1895

The *Faune de Madagascar's* monograph on terrestrial pulmonate gastropods (Fischer-Piette *et al.*, 1994) was a delayed, posthumous publication that overlooked some prior systematic changes within the Madagascan acavids. The most important of these were Mead's (1985) transfer of *Leucotaenius* Martens, 1860, from Achatinidae to Acavidae, and Emberton's (1990) reduction of *Eurystyla* Ancey, 1887, to a subgenus of Ampelita Beck, 1837; establishment of the new subgenera *Ampelita (Vesconis)* Emberton, 1990, and *A. (Xystera)* Emberton, 1990; and transfer of *Ampelita covani* (Smith, 1879) to Rhytididae (provisionally to *Rhytida* Albers, 1860).

Genus *Ampelita* Beck, 1837

Emberton's (1990) four subgenera of *Ampelita* were based on single anatomical differences, most of his species assignments to those subgenera were provisional and based on shells, and one such assignment was later contradicted by allozyme evidence (Emberton, 1995a). The genus *Ampelita* is seriously in need of revision. Such a project would be aided by the anatomical and frozen-tissue materials now available for many species (Emberton, unpublished, this paper). In the meantime, however, there is no point in trying to assign these new species to subgenera, except for the one *A. (Eurystyla)*.

Fischer-Piette *et al.* (1994:87-89, 147), in contrast, put *Ampelita* species into six phenetic groups–counting *A. (Eurystyla)* as the sixth–based on gross shell morphology. Despite some inconsistencies, these are useful for identification purposes, so have been followed here. Species are arranged alphabetically within Fischer-Piette *et al.*'s (1994) groups. Written definitions of the groups were sketchy and inconsistent, so are clarified and corrected here, based on included species. Except for Group 6, these groups have no taxonomic validity.

Group 1. Non-carinate, rounded periphery; low spired; umbilicus broad to narrow; peristome slightly to moderately reflected; no dorsal, spiral gutter.

Ampelita anjanaharibei sp. nov.
Fig. 1

HOLOTYPE. USNM 880350 (ex MBI 693.50DH and AH, ad shell and pulled body in alc): 14°44'S, 49°26'E: Madagascar: Anjanaharibe Sud Reserve, 1750 m. 20 Oct. 1995.

PARATYPE. AMS C.203526 (ex MBI 697.50DP, 1 ad): 14°44'S, 49°26'E: Madagascar: Anjanaharibe Sud Reserve, 1650 m. 20 Oct. 1995.

DESCRIPTION OF HOLOTYPE. Diameter 41.5 mm, height 24.0 mm, whorls 4.6. Body-whorl periphery rounded; suture moderately impressed, faintly crenulate; shoulder narrow, nearly a flat shelf. Umbilicus narrowly funneled, faintly enlarged by a rounded rim; width 5.4 mm (0.13 shell diameter). Aperture compressed-elliptical, height 11.7 mm, width 19.4 mm. Aperture downward deflection moderate, 0.1 whorl; face angle (relative to axis of coiling) 45°. Apertural lip reflection narrow to moderately wide, widest above; sharp edge rolled back and nearly under. Embryonic whorls 2.2; embryonic sculpture nearly smooth, then with minute pustules. Shell with a satin-like sheen, covered with small pustules, and with low, transverse growth lines and faint spiral lines. Color chocolate brown with rare, light-yellow, transverse-linear flecks, with a brownish yellow subsutural band that has a distinct upper edge and an indistinct lower edge, and with a narrow yellow-brown band on the umbilical rim.

VARIATION. Single paratype virtually identical to holotype, but with broken lip.

COMPARISONS. Very similar in size and shape to some *Ampelita subatropos* (Dautzenberg, 1894), but with a much narrower umbilicus and more rapid whorl expansion, and with a glossier, more rugose, less spirally engraved sculpture. Rounder whorls, tighter coiling, and smaller umbilicus than *A. gaudens* (Mabille, 1884). Completely lacks the spiral, dorsal gutter of other *Ampelitas* of the same size.

ETYMOLOGY. For Anjanaharibe Reserve, the type locality.

Ampelita ivohibei sp. nov.
Fig. 2

HOLOTYPE. USNM 880351 (ex MBI 1499.50DH and AH, ad shell and pulled body in alc): 24°34'S, 47°12'E: Madagascar: Ivohibe Forest, 570 m. 30 Oct. 1992.

PARATYPE. ANSP 401985 (ex MBI 1504.50DP, subadult shell): 24°34'S, 47°12'E: Madagascar: Ivohibe Forest, 400 m. 30 Oct. 1992.

DESCRIPTION OF HOLOTYPE (preserved in ethanol before the body was removed, therefore with some dissolution pits and breakage). Diameter 20.3 mm, height 10.8 mm, whorls 3.8. Body-whorl periphery rounded; suture very strongly and deeply impressed, simple; shoulder broad, flatly rounded. Umbilicus funneled, faintly enlarged by a slight, rounded rim; width 2.3 mm (0.11 shell diameter). Aperture compressed-elliptical, height 5.1 mm, width 8.9 mm. Aperture downward deflection moderate, <0.1 whorl; face angle (relative to axis of coiling) 55°. Apertural lip reflection narrowly and evenly rolled, thin edged. Embryonic whorls 2.2; embryonic sculpture apparently smooth. Shell somewhat glossy, with faint, irregular growth lines–otherwise smooth. Coloration consisting of transverse, irregular stripes of dark brown and ivory, overlain with four interrupted, peripheral bands of ivory.

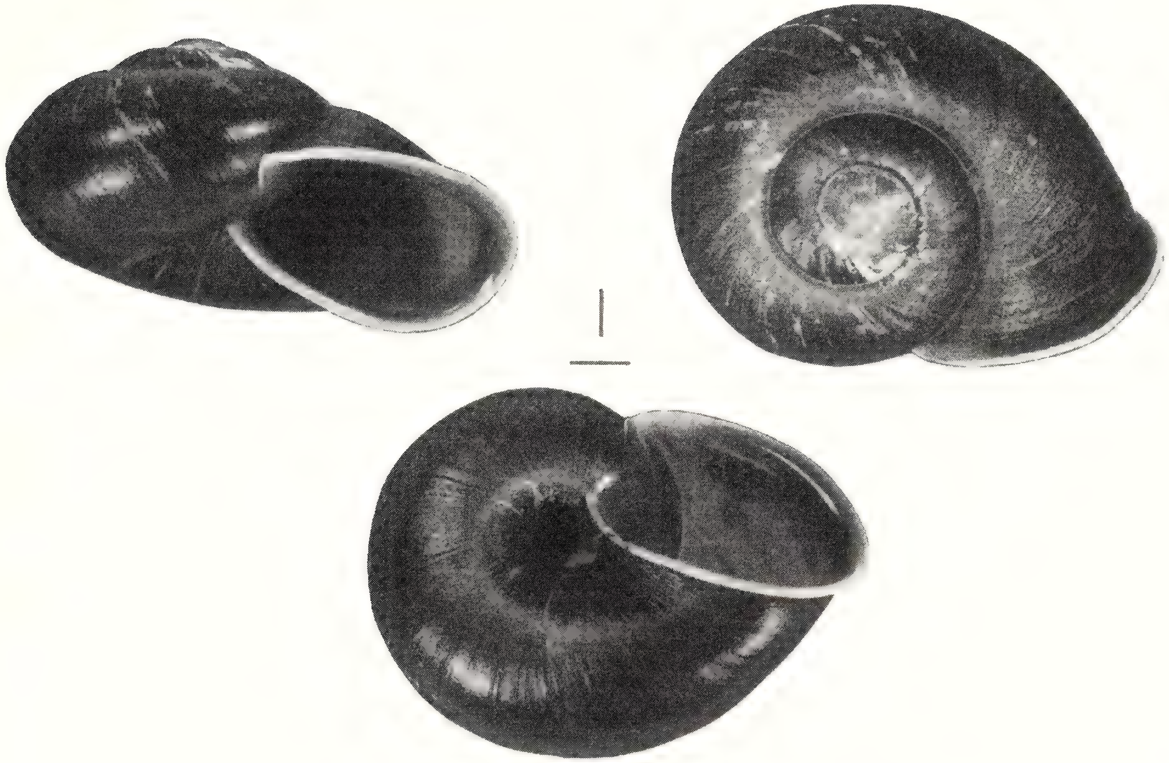
COMPARISONS. Unique for its tiny size and flat spire. Most similar to *Ampelita anosiana* Fischer-Piette, Blanc, Blanc, and Salvat, 1994, which is larger, with looser coiling, broader umbilicus, and sunken apex.

ETYMOLOGY. For Mount Ivohibe, also known as Mount Varabe, near the type locality.

Ampelita masoalae sp. nov.
Fig. 3

HOLOTYPE. USNM 880352 (ex MBI 309.50DH and AH, ad shell and pulled body in alc): 15°33'S, 50°0'E: Madagascar: Masoala National Park, 1000 m: hardwood rainforest with palms, pandanus, and tree moss. 26 Sep. 1995.

PARATYPES. AMS C.203484 (ex MBI 309.50DP, 1 ad): type locality. MNHN (ex MBI 309.50DP, 1 ad): type locality. ANSP 401986 (ex MBI 309.50DP, 1 ad): type locality. USNM 880353 (ex MBI 309.50DPR [1 juv], MBI 309.50DP [18 ad, 18 juv], and MBI 309.50AP [1 juv]): type locality. USNM 880400 (ex MBI 644.50DP, 4 ad, 2 juv & frag): 14°28'S, 49°34'E: Madagascar: Marojejy Reserve, incidental collecting along trail, 1050 m average elevation, 27 Sep. 1995.



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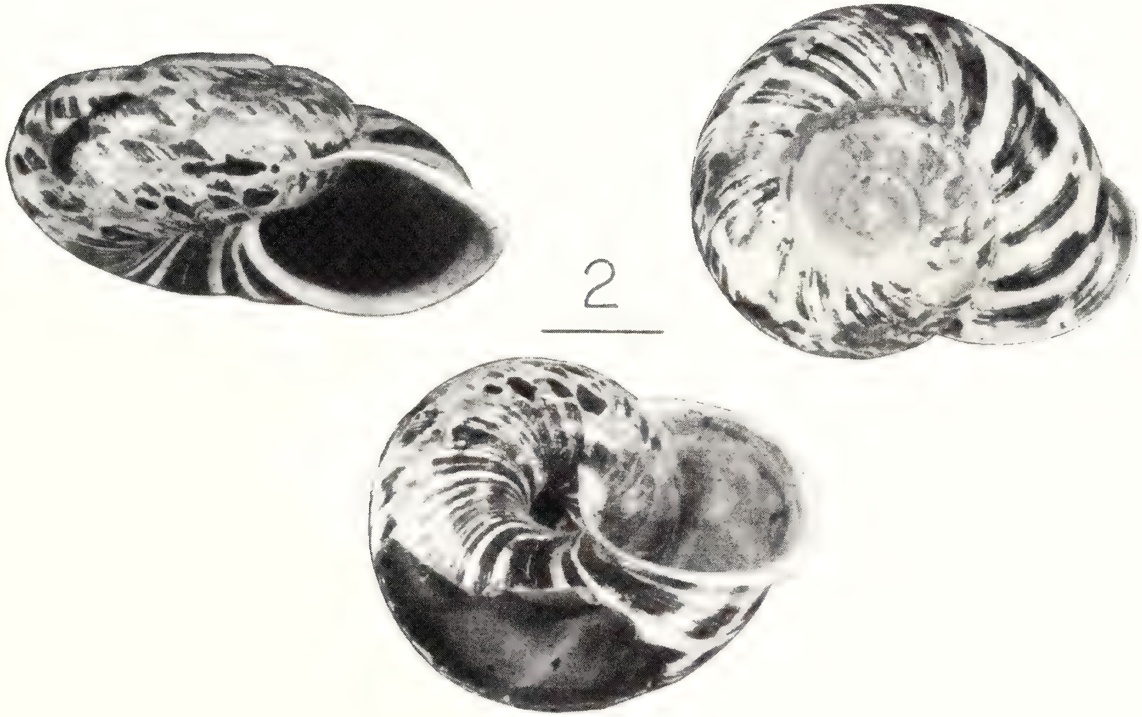

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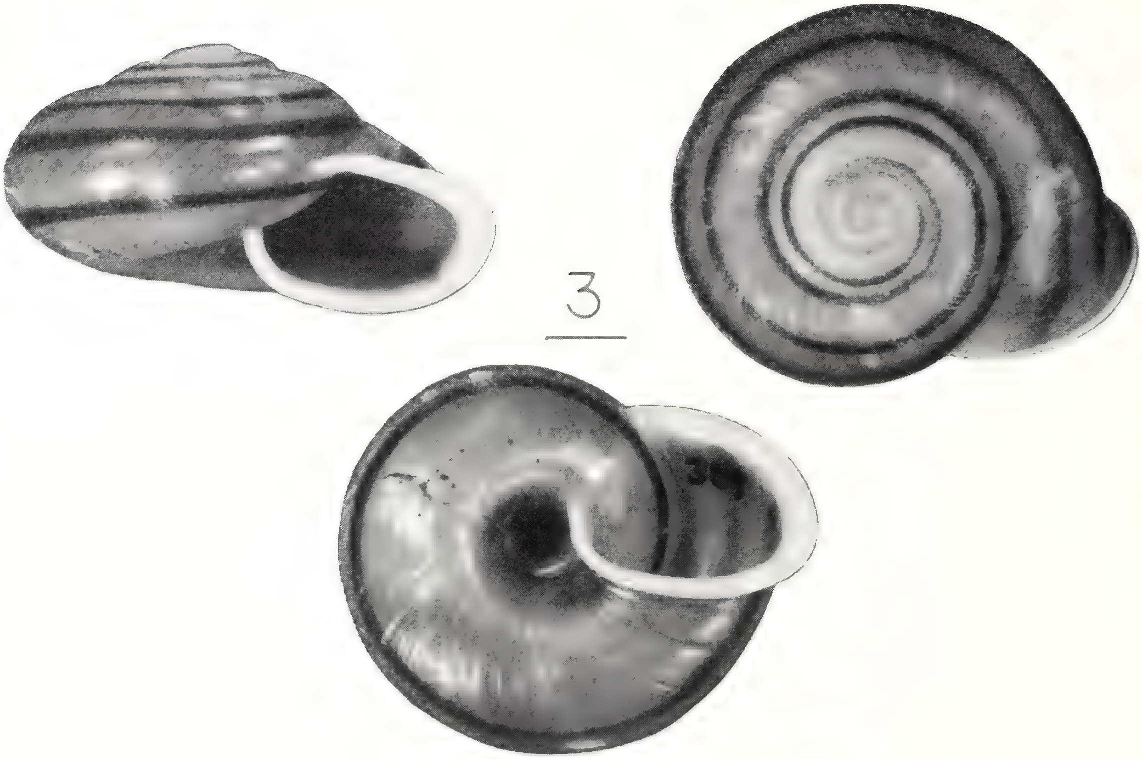
Figs. 1-2. Fig. 1. *Ampelita anjanaharibei* sp. nov. holotype. Fig. 2. *A. ivohibei* sp. nov. holotype. Scale bars 5 mm.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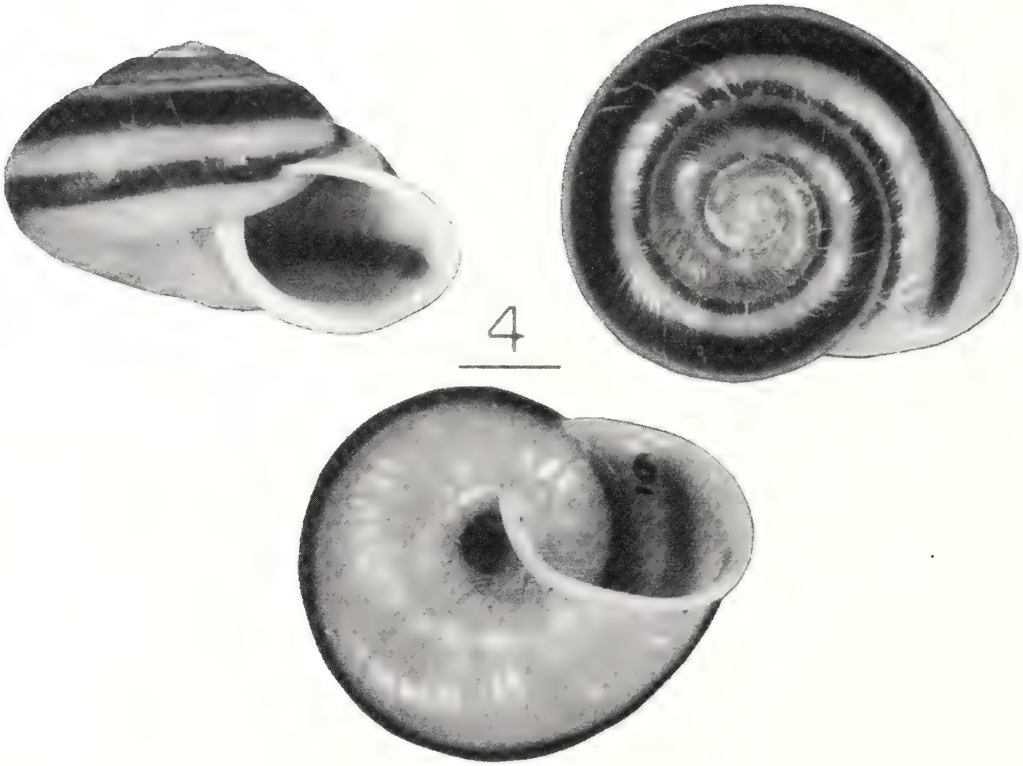
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Figs. 3-4. Fig. 3. *Ampelita masoalae* sp. nov. holotype. Fig. 4. *A. raxworthyi* sp. nov. holotype. Scale bars 5 mm.

DESCRIPTION OF HOLOTYPE (apex eroded). Diameter 30.0 mm, height 17.3 mm, whorls 4.6. Body-whorl periphery sharply rounded, with a faint trace of angulation; suture moderately impressed, simple; shoulder broadly rounded. Umbilicus broadly funneled, with only a slight trace of a rim; width 4.9 mm (0.16 shell diameter). Aperture compressed-elliptical, height 7.0 mm, width 11.7 mm. Aperture downward deflection moderate, 0.1 whorl; face angle (relative to axis of coiling) 40°. Apertural lip reflection narrowly to moderately wide, widest above; sharp edge rolled back and nearly under. Embryonic whorls 1.9. Shell with a faint sheen, bearing moderate-sized pustules, faint growth lines, and traces of dense spiral lines. Color dark yellow with three narrow bands of reddish brown; umbilicus with diffuse red-brown splotches; peristome and apertural interior white.

EMBRYONIC SCULPTURE (Paratype USNM 880353). Embryonic sculpture nearly smooth, but with faint transverse ribs and even fainter traces of spiral lines.

VARIATION. Shells from MBI 644 considerably flatter (H/D 0.50) and with a proportionally wider aperture (AW/D 0.42) and slightly more flared upper apertural lip; some paratypes lack color bands; adult diameters range 27.6-33.1 mm.

COMPARISONS. More tightly coiled than *Ampelita futura* Fischer-Piette and Garreau, 1965, the holotype of which has about three whorls (Fischer-Piette *et al.*, 1994:plate V, fig. 12), not four as described (Fischer-Piette *et al.*, 1994:90). Much more compressed whorls than *A. subsepulchralis* (Crosse, 1868). Differs from *A. consanguinea* (Férussac, 1851) and others of similar size and shape by its relatively wide, white peristome, in addition to other details.

ETYMOLOGY. For Masoala National Park, the type locality.

COMMENTS. Apparently restricted to high elevations. The Masoala and Marojejy populations thus appear to be extremely isolated and could be separate species or subspecies.

Ampelita raxworthyi sp. nov.

Fig. 4

HOLOTYPE. USNM 880354 (ex MBI 6.50DH and AH, ad shell and pulled body in alc): 24°46'S, 47°9'E: Madagascar: Forêt Sainte Luce, 10 m: coastal rainforest. 29 Jan. 1995.

PARATYPES. AMS C.203485 (ex MBI 6.50DP, 1 ad): type locality. MNHN (ex MBI 6.50DP, 1 ad): type locality. ANSP 401987 (ex MBI 6.50DP, 1 ad): type locality. USNM 880401 (ex MBI 6.50DP [4 ad, 6 juv] and MBI 7.50AP [1 juv, beat from vegetation, in alc]): type locality.

DESCRIPTION OF HOLOTYPE. Diameter 22.0 mm, height 14.5 mm, whorls 4.2. Body-whorl periphery rounded; suture moderately impressed, simple; shoulder narrow, nearly flat. Umbilicus a narrow pit, with only a slight trace of a rim; width 2.9 mm (0.13 shell diameter). Aperture compressed-elliptical, height 6.2 mm, width 9.1 mm. Aperture downward deflection moderate, 0.2 whorl; face angle (relative to axis of coiling) 50°. Apertural lip reflection narrowly and evenly rolled, thin edged. Embryonic whorls 2.1; embryonic sculpture nearly smooth in the first whorl, then with faint riblets. Shell slightly glossy, almost smooth, but with faint, irregular growth lines and obscure traces of spiral lines. Color yellow, with three dark reddish brown bands: one narrow subsutural, one broad supraperipheral, and one medium-broad subperipheral.

VARIATION. Smallest shell diameter 18.7 mm.

COMPARISONS. The small, compact, globose, umbilicate shape is approached only by *Ampelita parva* Fischer-Piette and Garreau, 1965, which is more domed and tightly coiled, and *A. petiti* Fischer-Piette, 1952, which is also more tightly coiled and has an unreflected upper peristome. *A. globulus* Fischer-Piette, Blanc, and Vukadinovic, 1974, bears some resemblance, but has a proportionally much larger aperture and looser coiling.

ETYMOLOGY. For Dr. Chris Raxworthy, herpetologist and biogeographer, who suggested collecting at the type locality.

Group 2 (no new species). Sub-carinate to non-carinate; low spired; umbilicus moderate to broad; peristome moderately reflected; no dorsal, spiral gutter.

Group 3. Carinate; low spired; umbilicus narrow or imperforate; peristome moderately reflected; no dorsal, spiral gutter.

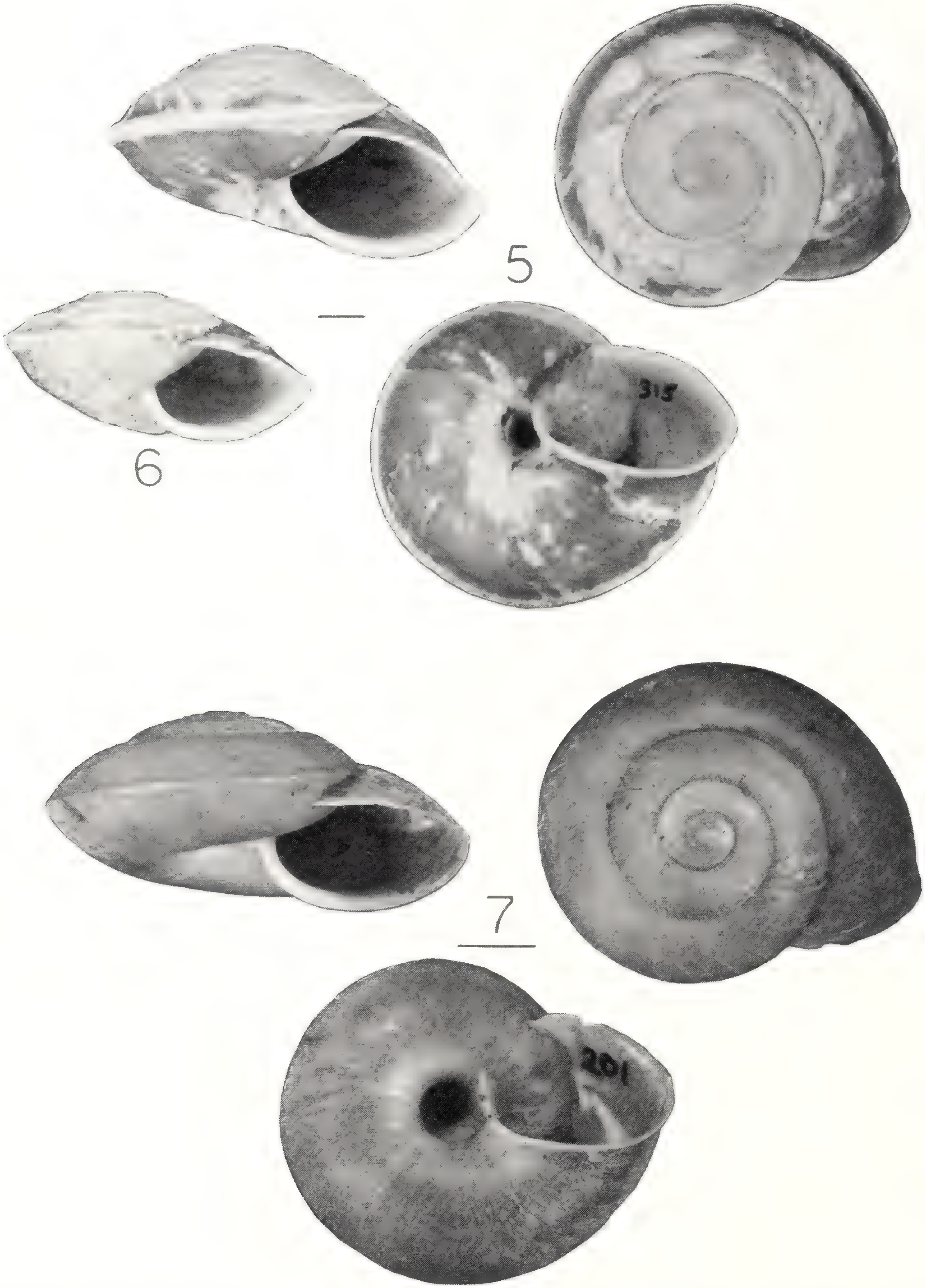
Ampelita ambanianae sp. nov.

Figs. 5, 6

HOLOTYPE. USNM 880355 (Fig. 5, ex MBI 315.50DH, ad shell): 15°40'S, 49°58'E: Madagascar: Masoala Peninsula: near Mount Ambaniana, trail to Andranobe, 0 to 300 m. 29 Sep. 1995.

PARATYPES. AMS C.203486 (ex MBI 315.50DP, 1 ad): type locality. MNHN (ex MBI 315.50DP, 1 ad): type locality. ANSP 401988 (ex MBI 315.50DP, 1 ad): type locality. USNM 880356 (Fig. 6, ex MBI 315.50DPR [ad shell]; and ex MBI 315.50DP [2 frag]): type locality.

DESCRIPTION OF HOLOTYPE (a weathered shell retaining about half of the body-whorl periostracum). Diameter 39.4 mm, height 23.2 mm, whorls 4.7. Body-whorl periphery carinate, carina wide, blunt-edged, and shallowly guttered above and below; suture shallowly impressed, bordered on either side by broad, shallow gutters; shoulder narrow, rounded, then dropping off steeply into a shallow gutter. Umbilicus narrow, steep-sided, faintly enlarged by a slightly angular rim; width 2.9 mm (0.07



Figs. 5-7. Figs. 5-6. *Ampelita ambanianae* sp. nov.: Fig. 5 holotype in three views; Fig. 6 paratype small adult in one view. Fig. 7. *A. analamerae* sp. nov. holotype. Scale bars 5 mm.

shell diameter). Aperture compressed-elliptical, height 10.8 mm, width 18.9 mm. Aperture downward deflection moderate, <0.1 whorl; face angle (relative to axis of coiling) 40°. Apertural lip reflection narrow, thin, incompletely rolled, narrow above, wider below. Shell sculpture consisting of parallel, slightly wavy, spiral lines; faint, broad growth ridges; and obscure, short, oblique cut marks that sometimes give a herringbone appearance. Color yellowish brown, sometimes with a faintly greenish cast.

VARIATION. Largest diameter 41.3 mm, most elevated shell H/D 0.60.

COMPARISONS. Differs from all others of Fischer-Piette *et al.*'s (1994) group 3 species by its two broad, dorsal, spiral channels, and by its round-edged, cord-like carina. In general shape it most resembles *Ampelita stumpfii* (Kobelt, 1880), but it has much looser coiling. Its size, coiling, color, and sculpture are similar to those of *A. lancula* (Férussac, 1821), from which it differs–in addition to its unique characters–by its more rounded aperture and greater pre-apertural downward deflection.

ETYMOLOGY. For Mount Ambaniana, near the type locality.

Group 4. Carinate; low spired; umbilicus broad; peristome narrowly reflected; no dorsal, spiral gutter.

Ampelita akoratsara sp. nov.
Fig. 8

HOLOTYPE. USNM 880357 (ex MBI 657.50DH and AH, ad shell and pulled body in alc): 14o°32'S, 49°42'E: Madagascar: near Marojejy Reserve, Ambatosorotra Mountain, 800 m: rainforest. 4 Oct. 1995.

PARATYPES. AMS C.203487 (ex MBI 648.50DP, 1 ad): 14°29'S, 49°33'E: Madagascar: Marojejy Reserve W, 805 m: rainforest; 28 Sep. 1995. MNHN (ex MBI 626.50DP, 1 ad): 14°20'S, 49°35'E: Madagascar: Marojejy Reserve W, incidental along trail, 950 m average elevation: rainforest; 24 Sep. 1995. ANSP 401989 (ex MBI 626.50DP, 1 ad): 14°20'S, 49°35'E: Madagascar: Marojejy Reserve W, incidental along trail, 950 m average elevation: rainforest; 24 Sep. 1995. USNM 880402, 880404 (ex MBI 626-631DP, 2 lots; total 2 ad, 2 juv): 14oS, 49oE: Madagascar: Marojejy Reserve W, incidental along trail, 950-1125 m: rainforest; Sep. 1995. USNM 880403, 880405, 880406 (ex MBI 657-674DP, 3 lots; total 2 ad, 1 juv): 14°32'S, 49°42'E: Madagascar: near Marojejy Reserve, Ambatosorotra Mountain, 800-940 m: rainforest; Oct. 1995. USNM 880407, 880408 (ex MBI 704-705DP, 2 lots; total 1 ad, 1 juv): 14°45'S, 49°28'E: Madagascar: Anjanaharibe Sud Reserve, 1100-1185 m: rainforest; 23 Oct. 1995.

DESCRIPTION OF HOLOTYPE. Diameter 27.6 mm, height 13.1 mm, whorls 4.0. Body-whorl periphery sharply carinate; suture shallowly impressed, simple; shoulder narrow, flattish. Umbilicus funneled, enlarged by a sharply angular rim; width 3.8 mm (0.14 shell diameter). Aperture compressed-elliptical, height 5.7 mm, width 11.7 mm. Aperture downward deflection great, 0.1 whorl; face angle (relative to axis of coiling) 60°. Apertural lip reflection narrow, thin, rolled, even. Embryonic whorls 2.0; embryonic sculpture nearly smooth, with slight, irregular pitting. Shell slightly glossy, with parallel spiral grooves transected by slightly fainter, more irregular growth lines. Ground color light brown; periphery and suture with a single thick band of white edged with dark brown; umbilicus slightly darker brown than ground color and edged with a band of light beige; peristome light beige.

VARIATION. Adult paratype diameters range 26.3-29.5 mm; less extreme preapertural downward deflections occur in several paratypes.

COMPARISONS. Among carinate, widely umbilicate *Ampelita* (Fischer-Piette *et al.*'s, 1994, group 4), most similar in size, shape, and general sculpture to *A. ranomafanae* sp. nov., but differs in its smooth (vs. pustulose) embryonic sculpture, its less shelved carina, and its greater pre-apertural deflection. Within group 4, *A. akoratsara* sp. nov. and *A. ranomafanae* sp. nov. share their spiral-groove sculpture only with *A. namerokoensis* Fischer-Piette, 1952, which is much more tightly coiled.

ETYMOLOGY. For the beautiful (Malagasy "tsara") shell (Malagasy "akora").

Ampelita analamerae sp. nov.
Fig. 7

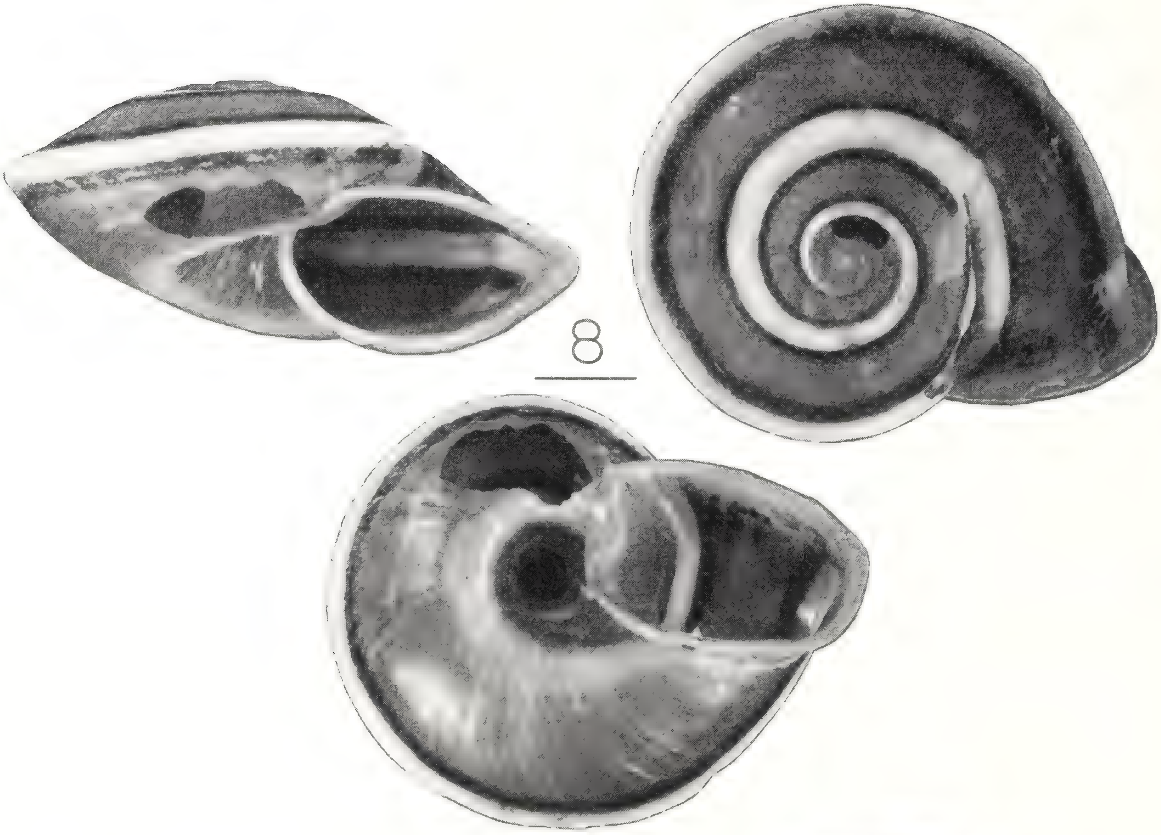
HOLOTYPE. USNM 880358 (ex MBI 201.50DH, ad shell): 12°44'S, 49°30'E: Madagascar: Analamera Reserve, 315 m: dry deciduous forest. 15 Jul. 1995.

PARATYPES. AMS C.203488 (ex MBI 201.50DP, 1 ad): type locality. MNHN (ex MBI 201.50DP, 1 ad): type locality. ANSP 401990 (ex MBI 201.50DP, 1 ad): type locality. USNM 880409 (ex MBI 201.50DP, 14 ad, 45 juv & frag): type locality.

DESCRIPTION OF HOLOTYPE. Diameter 27.2 mm, height 13.6 mm, whorls 4.8. Body-whorl periphery carinate; suture deeply impressed, very slightly crenulate; shoulder moderate, flat. Umbilicus funneled, faintly enlarged by a slightly angular rim; width 4.5 mm (0.17 shell diameter). Aperture compressed-elliptical, height 5.3 mm, width 11.3 mm. Aperture downward deflection strong, <0.1 whorl; face angle (relative to axis of coiling) 55°. Apertural lip reflection grading from no reflection at the suture, to narrowly reflected at the columella; edge sharp. Embryonic whorls 2.2; embryonic sculpture nearly smooth, with very faint riblets. Shell sculpture consists of closely spaced, even, sharp, transverse ridges that bear, when fresh, periostracal lamellar-like extensions. Color an even yellowish brown, lighter in the umbilicus.

VARIATION. Adult paratype diameters range 25.2-28.5 mm.

COMPARISONS. Among Fischer-Piette *et al.*'s (1994)



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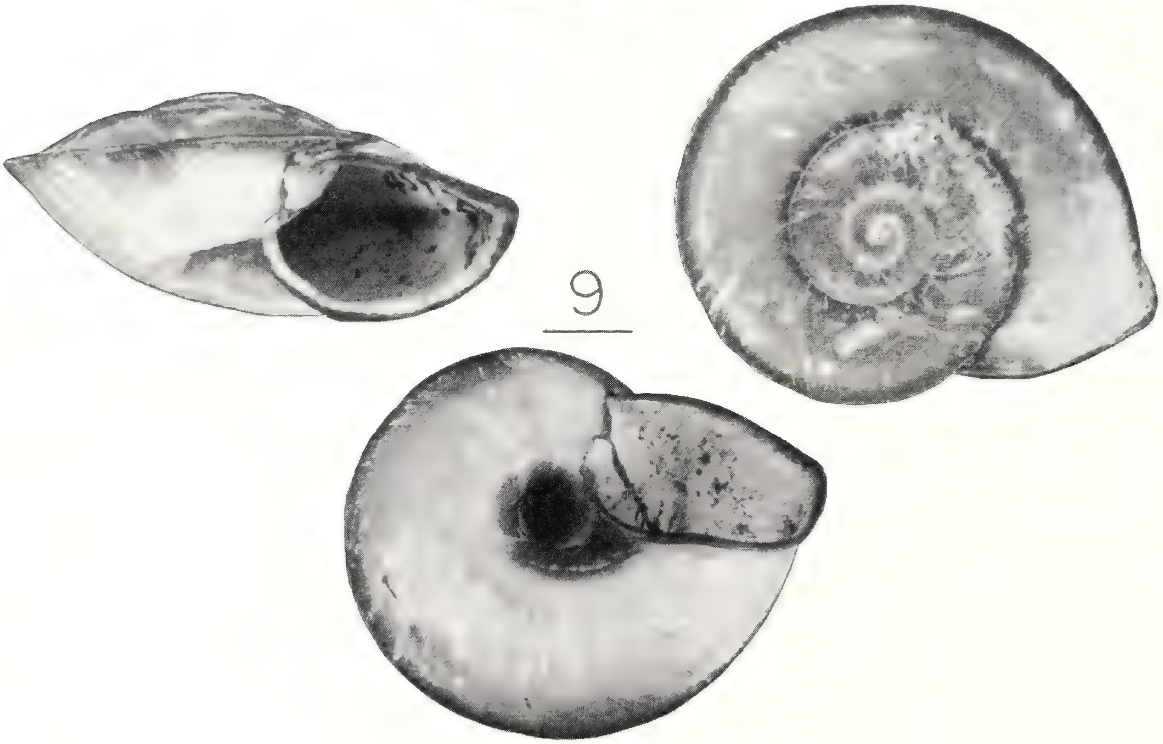

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Figs. 8-9. Fig. 8. *Ampelita akoratsara* sp. nov. holotype. Fig. 9. *A. ranomafanae* sp. nov. holotype. Scale bars 5 mm.

group 4 (carinate, widely umbilicate) species, unique for its distinctive sculpture. Most closely resembles *Ampelita namerokoensis* Fischer-Piette, 1952, in size and coiling tightness, but is higher spired and more narrowly umbilicate. In its strong pre-apertural deflection it resembles *A. bathiei* Fischer-Piette, 1952, and *A. akoratsara* sp. nov., but is much more tightly coiled, and its upper apertural lip is unreflected.

ETYMOLOGY. For Analamera Reserve, the type locality.

Ampelita ranomafanae sp. nov.

Fig. 9

HOLOTYPE. USNM 880359 (ex MBI 459.50DH, ad shell): 21°13'S, 47°25'E: Madagascar: Ambatolahy, adjacent to Ranomafana National Park, 850 m: rainforest. 9 Oct. 1995.

PARATYPE. USNM 880410 (ex MBI 459.50DP, 1 juv): type locality.

DESCRIPTION OF HOLOTYPE (originally with about a third of its periostracum, most of which has flaked off; shell somewhat eroded). Diameter 28.2 mm, height 12.9 mm, whorls 4.1. Body-whorl periphery very sharply carinate, upper surface of carina broad and nearly flat; suture shallowly impressed, simple; shoulder narrow, flatly rounded. Umbilicus steep-sided, enlarged by an angular, raised rim; width 4.4 mm (0.16 shell diameter). Aperture compressed-elliptical, height 7.0 mm, width 11.1 mm. Aperture downward deflection slight, <0.1 whorl; face angle (relative to axis of coiling) 45°. Apertural lip reflection narrow, thin, incompletely rolled, even in width. Embryonic whorls 2.0; embryonic sculpture apparently initially smooth, then pustulose. Shell slightly glossy, with parallel spiral grooves transected by slightly stronger growth lines; appearance almost beaded in places. Color yellowish brown, with carina, suture, and peristome a dark, purplish brown.

COMPARISONS. See comparisons under *Ampelita akoratsara* sp. nov.

ETYMOLOGY. For Ranomafana National Park, adjacent to the type locality.

Group 5. Non-carinate, rounded periphery; low spired; umbilicus broad; peristome broadly flared; with or without dorsal, spiral gutter.

Ampelita josephinae sp. nov.

Fig. 10

HOLOTYPE. USNM 880360 (ex MBI 357.50DH and AH, ad shell and pulled body in alc): 16°19'S, 49°46'E: Madagascar: W of Sahasoa, 330 m: hardwood rainforest. 21 Oct. 1995.

PARATYPES. AMS C.203489 (ex MBI 355.50DP, 1 ad): 16°19'S, 49°44'E: Madagascar: W of Mount Andaitra, 510 m: hardwood and pandanus; 19 Oct. 1995. MNHN (ex MBI 351.50DP, 1 ad): 16°19'S, 49°44'E: Madagascar: summit Mount Andaitra, 515 m: hardwood and pandanus; 18 Oct. 1995. ANSP 401991 (ex MBI 351.50DP, 1 ad): 16°19'S, 49°44'E: Madagascar: summit Mount Andaitra, 515 m: hardwood and pandanus; 18 Oct. 1995. USNM 880411-880420 (ex MBI 347-357DP, 10 lots; total 24 ad, 5 juv): 16°S, 49°E: Madagascar: Mount Andaitra area, 300-515 m; Oct. 1995.

DESCRIPTION OF HOLOTYPE. Diameter 37.2 mm, height 19.7 mm, whorls 4.1. Body-whorl periphery broadly rounded; suture moderately impressed, simple; shoulder broad, gently rounded. Umbilicus a pinhole, then rapidly expanding in the last half whorl; artificially enlarged by a rounded rim offset internally by two parallel grooves; width 2 mm (0.07 shell diameter). Aperture broadly auriculate, height 9.3 mm, width 15.5 mm. Aperture downward deflection strong, 0.1 whorl; face angle (relative to axis of coiling) 60°. Apertural lip very broadly and flatly reflected, wider above; edge sharp, rolled back. Embryonic whorls 2.2; embryonic sculpture nearly smooth, then with faint, interrupted, tranverse riblets. Shell with a satin-like sheen, covered with small, low pustules, and with faint, irregular growth lines. Color a dark, vivid yellow, with light-yellow flecks; apex and inner and outer peristome dark brownish purple; outer edge of peristome white; apertural interior white; the shell is white where peristome has flaked off.

VARIATION. All adult specimens very similar in size and shape.

COMPARISONS. Other than *Ampelita perampla* Dautzenberg, 1907 (as redefined by Fischer-Piette *et al.*, 1994), this is the only flared-lip *Ampelita* without a conspicuous dorsal, spiral gutter. It differs from *A. perampla* in its more rapid post-embryonic whorl expansion (diameter of first three whorls 12.8 mm vs. 9.9 mm), its lower spire, its fainter and sparser pustulate sculpture, its proportionally smaller, less flared aperture, and its subtly different umbilicus.

ETYMOLOGY. For Josephine Djaohasara Emberton, the author's wife, who helped him collect this lovely species.

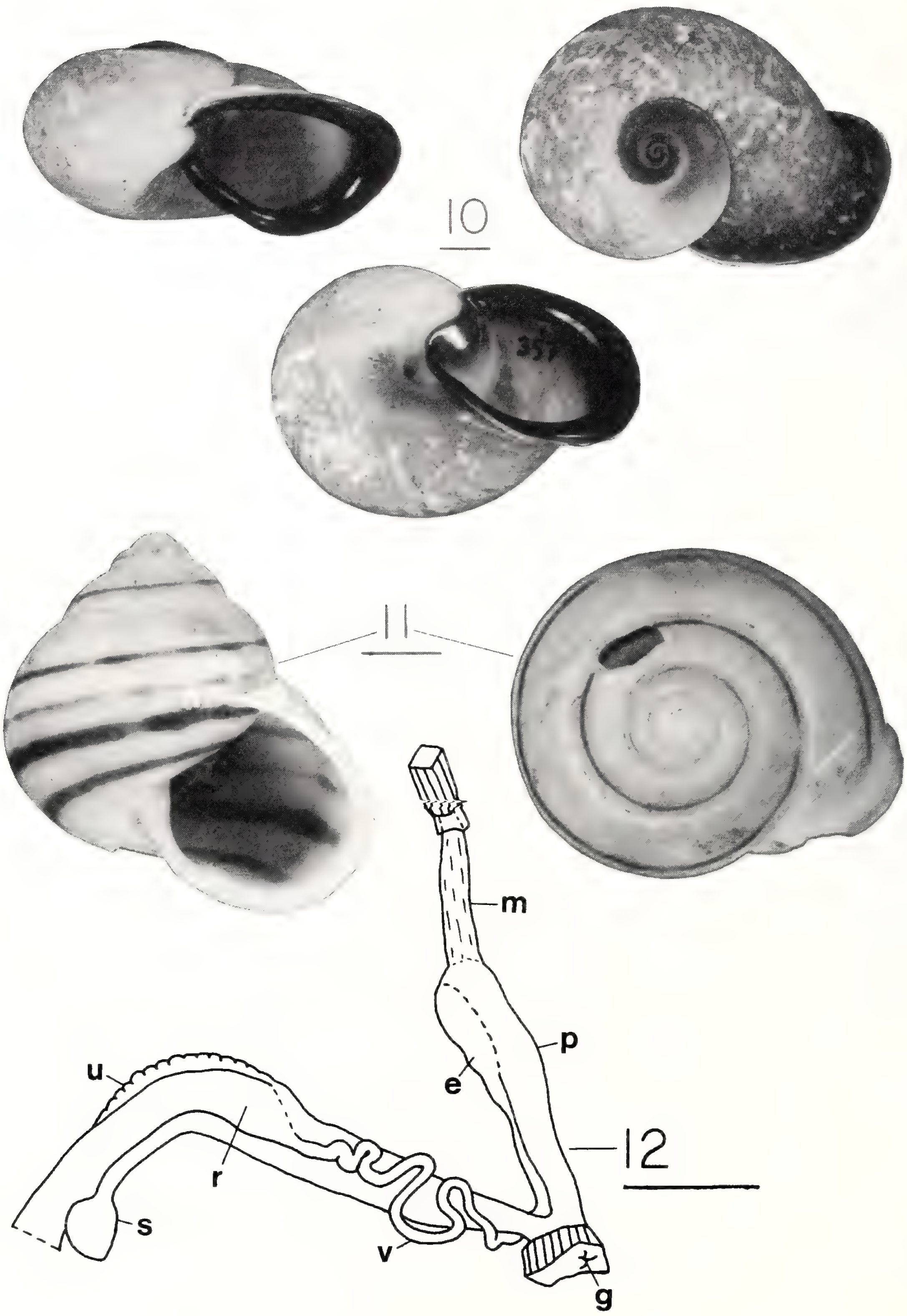
Group 6 = *Ampelita (Eurystyla)* Ancey, 1887. Non-carinate, rounded periphery; high spired; imperforate to creviced; peristome moderately reflected; no dorsal, spiral gutter.

Ampelita (Eurystyla) griffithsi sp. nov.

Figs. 11, 12

HOLOTYPE. USNM 880361 (ex MBI 250.50DH and AH, ad shell and pulled body in alc): 19°8'S, 44°49'E: Madagascar: S Bemaraha Reserve, 80 m: river gallery forest. 16 June 1995.

PARATYPES. AMS C.203490 (ex MBI 247.50DP, 3 ad, 6 juv and frag): 20°3'S, 44°39'E: Madagascar: Kirindy, 40 m: river gallery forest; 15 June 1995. AMS C.203491 (ex MBI 249.50DP,



Figs. 10-12. Fig. 10. *Ampelita josephinae* sp. nov. holotype. Figs. 11-12. *A. (Eurystyla) griffithsi* sp. nov. holotype: Fig. 11 shell in two views; Fig. 12 genitalia (abbreviations: e epiphallus, g genital pore, m penial retractor muscle, p penis, r prostate, s spermatheca, u uterus, v vas deferens). Scale bars 5 mm.

4 ad, 5 juv and frag): 19°8'S, 44°50'E: Madagascar: Bemaraha Reserve: river gallery forest; 16 June 1995. AMS C.203492 (ex MBI 494.50DP, 2 ad, 1 juv): 18°45'S, 44o°45'E: Madagascar: N Bemaraha Reserve, 280 m: semideciduous forest; 29 June 1996. AMS C.203493 (ex MBI 495.50DP, 1 ad, 2 juv): 18°47'S, 44°47'E: Madagascar: N Bemaraha Reserve, 300 m: deciduous scrub; 29 June 1996. MNHN (ex MBI 249.50DP, 1 ad): 19°8'S, 44°50'E: Madagascar: Bemaraha Reserve: river gallery forest; 16 June 1995. ANSP 401992 (ex MBI 247.50DP, 1 ad): 20°3'S, 44°39'E: Madagascar: Kirindy, 40 m: river gallery forest; 15 June 1995. Nationnal Natuurhistorisch Museum, Leiden, the Netherlands 59149 (1 ad in alcohol): 19°9'S, 44°49'E: Madagascar: Bemaraha Reserve. USNM 880421-880426 (ex MBI 247-251DP and 488-495DP, 6 lots; total 10 ad, 21 juv and frag): 20°3'S, 44°39'E: Madagascar: Kirindy and Bemaraha Reserve; 1995, 1996.

DESCRIPTION OF HOLOTYPE. SHELL (a very thin, brownish yellow periostracum remains on some paratypes but is lost from the holotype). Diameter 22.2 mm, height 23.6 mm, whorls 4.2. Body-whorl periphery broadly rounded; suture moderately impressed, simple; shoulder falling off steeply, broadly rounded. Umbilicus a crevice; width 0.8 mm (0.04 shell diameter). Aperture oval, height 10.6 mm, width 10.8 mm. Aperture downward deflection slight, 0.2 whorl; face angle (relative to axis of coiling) 30°. Apertural lip reflection narrowly and evenly rolled, expanded at the columellar insertion. Embryonic sculpture smooth, then with faint, wavy riblets. Shell with a faint sheen, covered with small pustules, and with low growth lines and traces of spiral lines. Color whitish yellow, with four thin, dark-brown bands.

GENITALIA. Genital pore far forward, just behind the right tentacle. Right tentacular retractor muscle passes between the penis and the vagina. Atrium small, without appendages. Penis 10 mm in length, about six times as long as wide. Penial retractor muscle insertion on the penial apex, origin on the interior body wall near the junction of the left mantle collar, origin apparently enveloped by a small, thin sheath. Vas deferens long, convoluted, bound to the penis by connective-tissue strands. Epiphallus bulbous, thin-walled, adherent to the penis. Vagina about two-thirds the length of the penis. Lower spermathecal (bursal, gametolytic) duct slightly swollen; upper duct slender, opening into a small, globular spermatheca (bursa copulatrix, gametolytic gland); spermatheca plus duct about the same length as the penis.

VARIATION. Adult shells more elevated at MBI 247 and 249 (H/D 1.1), smaller at MBI 494 and 495 (smallest diameter 19.2 mm); all paratypes lack columellar apertural node.

COMPARISONS. In size and shape most similar to *Ampelita (Eurystyla) viridis* (Deshayes, 1838), but with a pronouncedly sharper spire, tighter coiling, exposed umbilicus, and much different sculpture. In penial morphology, very similar to *A. (E.) cerina* (Morelet, 1877) (Fischer-Piette and Garreau de Loubresse, 1965:fig. 20).

ETYMOLOGY. For Owen Griffiths, collector of this species.

COMMENTS. Discovery of *Ampelita (Eurystyla) griffithsi* sp. nov. greatly expands the geographical, ecological, and morphological ranges of its subgenus.

Genus *Clavator* von Martens in Albers, 1860

Clavator griffithsjonesi sp. nov.

Figs. 13, 14

HOLOTYPE. USNM 880362 (Fig. 14, ex MBI 483.50DH, ad shell; and ex MBI 483.50AH (pulled body in alc): 18°1'S, 44°31'E: Madagascar: N Bemaraha Reserve. 22 June 1996.

PARATYPES. AMS C.203494 (ex MBI 483.50DP, 2 ad, 2 juv): type locality. MNHN (ex MBI 483.50DP, 1 ad): type locality. ANSP 401993 (ex MBI 483.50DP, 1 ad): type locality. USNM 880363 (Fig. 13, ex MBI 483.50DPR, juv shell; and ex MBI 483.50DP [2 juv & frag] and AP [1 ad in alc]): type locality. USNM 880427 (ex MBI 484.50DP, 1 ad): 18°3'S, 44°31'E: Madagascar: N Bemaraha Reserve; 23 June 1996.

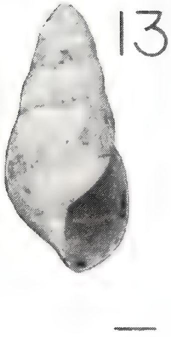
DESCRIPTION OF HOLOTYPE (apex broken). Diameter 32.7 mm, height 91.4 mm, whorls 9.6 (estimated). Body-whorl periphery flatly rounded; suture strongly impressed, very slightly guttered, slightly crenulate; shoulder narrow, steeply sloped. Umbilicus a narrow crevice; width 0.3 mm (0.01 shell diameter). Aperture slightly auriculate, height 28.3 mm, width 15.5 mm. Aperture downward deflection slight, <0.01 whorl; face angle (relative to axis of coiling) 10°. Apertural lip reflection slight, thick, and even but broadened at the columella. Shell somewhat glossy, with fine, closely spaced riblets and irregularly spaced growth-interruption lines; a faint subsutural line sometimes detectable. Color light yellow-brown to almost white, with occasional streaks and splotches of darker, somewhat reddish brown.

EMBRYONIC-SHELL CHARACTERS (Paratype USNM 880363). Embryonic whorls 4.2; embryonic sculpture nearly smooth in the first whorl; subsequent whorls with closely spaced riblets; subsuture can be slightly corded.

VARIATION. Only slight variation in height and apertural shape.

COMPARISONS. Unique in its shape and strongly ribbed sculpture; superficially somewhat like a giant *C. moreleti* (Férussac, 1851); much more tightly coiled and straight-sided than *C. clavator* (Petit de la Saussaye, 1844), *C. grandidieri* (Crosse and Fischer, 1868), and *C. anteclavator* Fischer-Piette, 1963, which it can resemble somewhat in color.

ETYMOLOGY. For Owen Griffiths (Bioculture Mauritius) and Dr. Carl Jones (Jersey/Mauritius Wildlife Appeal Fund), collectors of this species.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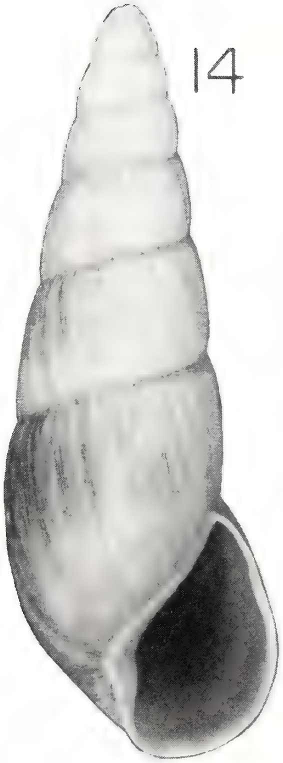
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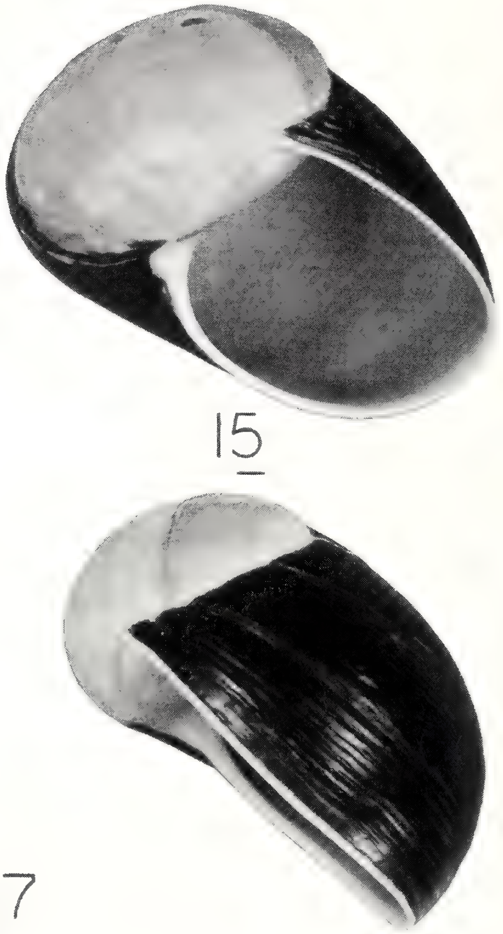
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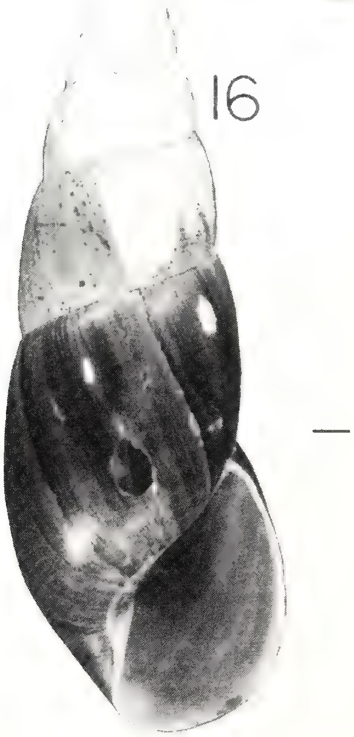
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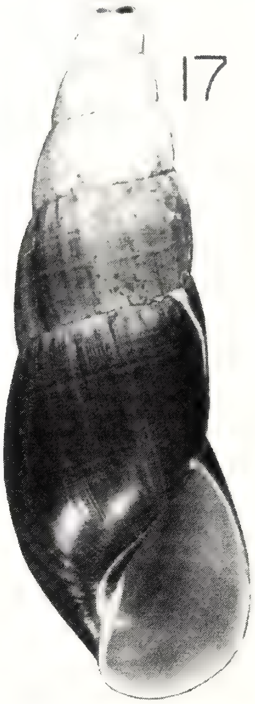
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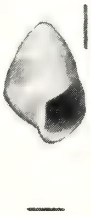
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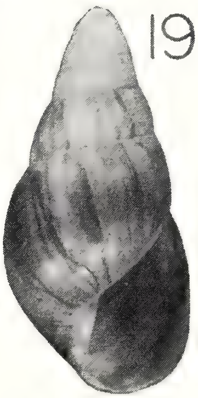
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Figs. 13-19. Figs. 13-14. *Clavator griffithsjonesi* sp. nov.: Fig. 13 juvenile paratype with complete apex; Fig. 14 holotype adult with broken apex. Fig. 15. *Helicophanta gargantua* sp. nov. holotype in two views. Figs. 16-19. *Clavator masoalae* sp. nov.: Fig. 16 holotype adult with naturally truncate apex; Fig. 17 paratype adult with truncate apex; Fig. 18 paratype small juvenile with complete apex; Fig. 19 paratype large juvenile with broken apex. Scale bars 5 mm.

Clavator masoalae sp. nov.

Figs. 16-19

HOLOTYPE. USNM 880364 (Fig. 16, ex MBI 309.51DH, ad shell; and ex MBI 309.51AH, ad body, partially decayed, in alc): 15°33'S, 50°0'E: Madagascar: Masoala National Park, 1000 m: hardwood rainforest with palms, pandanus, and tree moss. 26 Sep. 1995.

PARATYPES. AMS C.203495 (ex MBI 309.51DP, 1 ad, 1 juv): type locality. MNHN (ex MBI 309.51DP, 1 ad, 1 juv): type locality. ANSP 401994 (ex MBI 309.51DP, 1 ad, 1 juv): type locality. USNM 880365 (Fig. 17, ex MBI 744.50DPR, 1 ad; and ex MBI 744.50AP, pulled body in alc): 16°46'S, 49°8'E: Madagascar: Ambatovaky Reserve, 1025 m: rainforest with pandanus; 21 Nov. 1995. USNM 880366 (Fig. 19, ex MBI 296.50DPR, 1 juv; and ex MBI 296.50AP, 1 juv and pulled juv body in alc): 15°47'S, 50°3'E: Madagascar: Masoala National Park, 310-450 m; 19 Sep. 1995. USNM 880367 (Fig. 18, ex MBI 605.50DPR, 1 juv): 14°26'S, 49°45'E: Madagascar: Marojejy Reserve, 1200 m: rainforest. ; 16 Sep. 1995. USNM 880428 (ex MBI 308.50DP [1 ad, 2 juv] and AP (1 ad, partially decayed, in alc): 15°33'S, 50°0'E: Madagascar: Masoala National Park, 680-1000 m; 26 Sep. 1995. USNM 880429 (ex MBI 309.51DP [5 ad, 7 juv] and AP (1 juv in alc): type locality. USNM 880430-880434 (ex MBI 593-644DP, 5 lots; total 5 ad, 18 juv): 14°S, 49°E: Madagascar: Marojejy Reserve, 900-1350 m: rainforest; Sep. 1995. USNM 880435-880440 (ex MBI 741-749DP, 6 lots; total 9 ad, 5 juv): 16°S, 49°E: Madagascar: Ambatovaky Reserve, 870-1055 m; Nov. 1995.

DESCRIPTION OF HOLOTYPE. Diameter 36.0 mm, height 105.9 mm, apex naturally truncated, whorls estimated at approximately 9.4. Body-whorl periphery flatly rounded; suture strongly impressed, slightly crenulate; shoulder narrow, steeply sloped. Umbilicus imperforate, with a translucent callus. Aperture slightly auriculate, height 35.2 mm, width 19.3 mm. Aperture downward deflection slight, <0.01 whorl; face angle (relative to axis of coiling) 10°. Apertural lip reflection slight, thick, even but broadened at the columella. Shell somewhat glossy, with frequent, low, fairly regular growth lines crossed by fairly regularly spaced spiral lines. Color of embryonic whorls light yellow-brown; later whorls becoming progressively more reddish-brown with darker transverse streaks; body whorl reddish brown; apertural lip white; apertural interior bluish white.

EMBRYONIC-SHELL CHARACTERS (Paratypes USNM 880366 and 880367). Embryonic whorls 4.9; embryonic sculpture of faint riblets in the first whorl; subsequent whorls with closely spaced riblets cut by spiral lines to produce a strongly beaded appearance.

VARIATION. Aperture wide in some specimens, minimum H/W 1.6; shells from Ambatovaky Reserve are slenderer (Fig. 17).

COMPARISONS. In form, color, and sculpture, superficially resembles *C. eximius* (Shuttleworth, 1852); *C. dingeoni* Fischer-Piette, Blanc, and Salvat, 1975; *C. pauliani* Fischer-Piette, 1963; and *C. bathiei* Fischer-Piette, 1963; but conspicuously tighter coiling than the first three and slightly tighter coiling, a more acute apex, a proportionally larger aperture, and stronger spiral sculpture than *C. bathiei.*

ETYMOLOGY. For Masoala National Park, the type locality.

Genus *Helicophanta* Férussac, 1821

Helicophanta gargantua sp. nov.

Fig. 15

HOLOTYPE. USNM 880368 (ex MBI 1402.50DH and AH, ad shell and pulled body in alc): 22°4'S, 46°54'E: Madagascar: near Andringitra Reserve. 3 Oct. 1992.

PARATYPES. AMS C.203758 (ex MBI 1402.50DP, 1 ad): type locality. MNHN (ex MBI 1402.50DP, 1 ad): type locality. ANSP 401995 (ex MBI 1401.50DP, 1 ad): type locality. pre-1992. ANSP 401996 (ex MBI 1402.50DP, 17 ad, 11 juv): type locality. ANSP A19000 (ex MBI 1402.50AP, 2 ad, 2 juv): type locality. USNM 880369 (ex MBI 1402.50DPR, juvenile broken shell): type locality.

DESCRIPTION OF HOLOTYPE (apex eroded, so juvenile paratype USNM 880369 consulted to confirm embryonic sculpture). Diameter 90.9 mm, height 79.3 mm, whorls 4.5. Body-whorl periphery broadly rounded; suture strongly impressed, simple; shoulder steeply sloped, flattened. Umbilicus imperforate, with a thick white callus. Aperture broadly elliptical, height 60.6 mm, width 55.8 mm. Aperture downward deflection extreme, 0.5 whorl; face angle (relative to axis of coiling) 40°. Apertural lip reflection slight, thick, even. Embryonic whorls 3.5; embryonic sculpture consisting of elongate, transverse pustules arrayed as growth lines. Shell glossy, with strong, nearly regular growth lines; faint, supraperipheral spiral cords; and small, obscure, subperipheral, oblique cut marks. Color very dark brown, with reddish undertint; spiral cords darker brown; light beige where periostracum eroded; apertural lip and interior white.

COMPARISONS. Most similar in form and sculpture to *H. gloriosa* (Pfeiffer, 1856), from which it differs in its tighter initial coiling (10.0 vs 11.2 mm diameter of first 2.5 whorls) but much larger embryonic shell (3.6 vs. 3.2 whorls) and adult shell.

ETYMOLOGY. For the very large (Rabelais's fictional giant, Gargantua) shell size.

ACKNOWLEDGMENTS

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ANGAP. Ranomafana National Park Project gave logistic support. Marojejy and Anjanaharibe expeditions were led by Dr. Tim Pearce; Bemaraha by Owen Griffiths; Ivohibe by Ruffin Arijaona; Ambatolahy-Ranomafana National Park by Roger Randalana; and the remainder by the author. Major assistance on one or more of the Masoala, Analamera, Sahasoa-Andaitra, Sainte-Luce, and Andringitra expeditions was provided by Max Felix Rakotomalala *(requiescat in pace)*, Tim Pearce, Jean Rakotoarison, and Ruffin Arijaona. Many local Malagasy guides and collectors also rendered service. Dr. A. J. de Winter lent the Netherlands' Nationnal Natuurhistorisch Museum's specimen of *Ampelita (Eurystyla) griffithsi* sp. nov.

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Edentulina of Madagascar (Pulmonata: Streptaxidae)

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Abstract: *Edentulina* Pfeiffer, 1856, contains some of the largest species of the diverse, carnivorous, land-snail family Streptaxidae and seems to be restricted to tropical Africa, Madagascar, and some other Indian-Ocean islands. Based on extensive collections made in 1992-1995, and on the 1994 *Faune de Madagascar* pulmonate monograph, 11 native Madagascan species of *Edentulina* Pfeiffer, 1856, can be recognized: *E. ambongoaboae* sp. nov.; *E. ambra* sp. nov.; *E. analamerae* sp. nov.; *E. ankaranae* sp. nov.; *E. antankarana* sp. nov.; *E. arenicola* (Morelet, 1860); *E. battistinii* Fischer-Piette, F. Blanc, and Salvat, 1975; *E. bemarahae* sp. nov.; *E. bobaombiae* sp. nov.; *E. florensi* sp. nov.; *E. minor* (Morelet, 1851); *E. nitens* (Dautzenberg, 1895); and *E. rugosa* sp. nov.

Five species are synonymized under *Edentulina minor*: *E. alluaudi* (Dautzenberg, 1895); *E. gaillardi* Fischer-Piette and Bedoucha, 1964; *E. intermedia* (Morelet, 1851); *E. montis* Fischer-Piette, F. Blanc, and Salvat, 1975; and *E. stumpfii* Kobelt, 1904. Three species are transferred to a new genus described in a separate paper: *Edentulina (?) glessi* Fischer-Piette, Blanc, Blanc, and Salvat, 1994; *E. (?) metula* (Crosse, 1881); and *E. (?) simeni* Fischer-Piette, Blanc, Blanc, and Salvat, 1994. A Seychellean species, *E. dussumieri* (Dufo, 1840), is deleted from Madagascar's faunal list.

A dichotomous key is given to the native species plus the reportedly introduced *Edentulina ovoidea* (Bruguiere, 1792). Conchological descriptions are given of all native species.

Many promising regions of Madagascar remain uncollected. Further exploration should yield additional new species of *Edentulina*.

Key words: Gastropoda, Stylommatophora, land snails, taxonomy, shell variation

This paper is the first in a series on the conchological identification of Madagascar's lesser-known land-snail groups, based on extensive collections made in 1992-1995, and supplemental to the *Faune de Madagascar* monographs of Fischer-Piette *et al.* (1993, 1994).

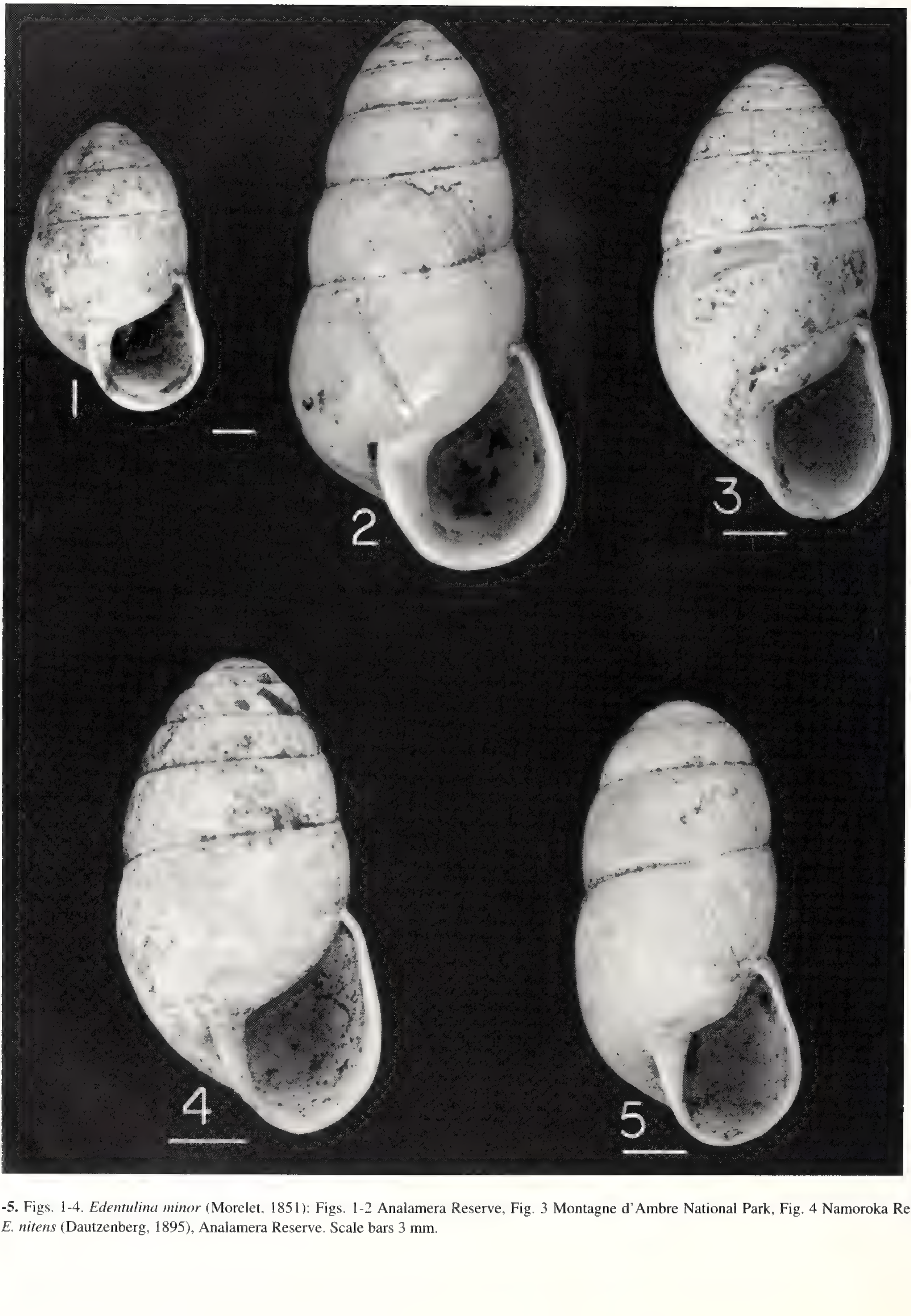
Edentulina Pfeiffer, 1856, contains some of the largest species of the diverse, carnivorous family Streptaxidae and seems to be restricted to Africa, Madagascar, and some other Indian-Ocean islands (Zilch, 1959-1960:563). The pulmonate gastropod volume of the *Faune de Madagascar* (Fischer-Piette *et al.*, 1994) summarized knowledge of that island's *Edentulina*, listing 14 species. Among those species, three were listed as tenuous (Fischer-Piette *et al.*, 1994) and have subsequently been transferred to a new genus (Emberton and Pearce, in press): *E. (?) glessi* Fischer-Piette, Blanc, Blanc, and Salvat, 1994; *E. (?) metula* (Crosse, 1881); and *E. (?) simeni* Fischer-Piette, Blanc, Blanc, and Salvat, 1994.

METHODS AND MATERIALS

Materials were collected in 1992-1995. Identifications and comparisons were made using Fischer-Piette *et al.* (1994) and available collections. Authors and dates of species are given as in Richardson (1988). Whorl counts were made in apical view by the widely used method of detecting the earliest sutural notch (using incidental lighting at 40x), extrapolating that notch as a line tangent to and projecting past the suture's initial right-hand curve, counting off whorls as that line crosses successive sutures, and rounding the final fraction of a whorl—ending at the suture's end, regardless of any distortion due to apertural-lip reflection—to the nearest tenth (Emberton, 1985:fig. 1; 1989). Measurements were made using an ocular micrometer and, rarely, vernier calipers. As an index of coiling tightness, the number of whorls was divided by the natural logarithm (ln) of the shell's height. Because of Madagascar's continuing environmental crisis and the urgency of providing data to conservationists and systematists, only conchological descriptions are given here.

KEY TO SPECIES

1a. Subsutural spiral cord present 2
1b. Subsutural spiral cord absent 3
2a. Sculpture smooth. . . . . *ovoidea*
(Fischer-Piette *et al.*, 1994:plate IV, figs. 4, 5)
2b. Sculpture ribbed. . . . . *minor* (Figs. 1, 2, 3, 4)



Figs. 1-5. Figs. 1-4. *Edentulina minor* (Morelet, 1851): Figs. 1-2 Analamera Reserve, Fig. 3 Montagne d'Ambre National Park, Fig. 4 Namoroka Reserve. Fig. 5. *E. nitens* (Dautzenberg, 1895), Analamera Reserve. Scale bars 3 mm.

3a. Sculpture smooth or with only subsutural traces of ribs 4
3b. Sculpture weakly to strongly ribbed 6

4a. No preapertural deflection of body whorl . . . . *nitens* (Fig. 5)
4b. Upward preapertural deflection 5

5a. Diameter of first 1.5 whorls about 2.3 mm, no trace of subsutural ribs . . . . *ambongoaboae* (Fig. 6)
5b. Diameter of first 1.5 whorls 1.7-1.9 mm, trace subsutural ribs present . . . . *ankaranae* (Fig. 7)

6a. Ribs faint to weak 7
6b. Ribs strong to moderate 8

7a. No preapertural deflection of body whorl, coiling loose (whorls/ln height about 2.3) . . . . *ambra* (Fig. 8)
7b. Upward preapertural deflection, coiling tight (whorls/ln height about 2.8). . . . . *analamerae* (Fig. 12)

8a. Ribs and sutural crenulation very strong, embryonic whorls smooth 9
8b. Ribs moderate and sutural crenulation weak to moderate, embryonic whorls sculpted with riblets. 10

9a. Coiling tight (whorls/ln height about 2.7-3.0), shell barrel-shaped to ovate, sutures fairly deeply impressed . . . . *arenicola* (Figs. 9, 10)
9b. Coiling loose (whorls/ln height about 2.5), shell bullet-shaped, sutures shallowly impressed . . . . *rugosa* (Fig. 11)

10a. Diameter of first 1.5 whorls 1.6-1.9 mm 11
10b. Diameter of first 1.5 whorls 2.2-2.3. 12

11a. Aperture narrow (height/width 1.3) and small (0.4 shell width) . . . . *antankarana* (Fig. 13)
11b. Aperture broader (height/width 1.0-1.2) and larger (0.5-0.6 shell width) . . . . *bemarahae* (Figs. 15, 16, 17, 18)

12a. Shape fusiform-oval, never any pre-apertural duplicate peristome(s) . . . . *battistinii* (Fig. 14)
12b. Shape pyramidal, pre-apertural duplicate peristome(s) often present. . . . . *florensi* (Figs. 19, 20)

SYSTEMATICS

To aid users seeking only to verify a previous identification, species descriptions are ordered alphabetically as in the Abstract. Higher classification follows Vaught (1989). Type materials are placed in the United States National Museum, Washington, D.C. (USNM); the Australian Museum, Sydney (AMS); the Muséum national d'Histoire naturelle, Paris (MNHN, which does not assign catalog numbers to types); and the Academy of Natural Sciences of Philadelphia (ANSP). Prior, working catalog numbers of the Molluscan Biodiversity Institute (MBI) are also given, because they provide reference to an ecological database available on request. MBI catalog numbers consist of station number, species reference number within that station, D (dry) or A (alcohol-preserved), and when appropriate H (holotype), P (paratype), or R (representative). As an aid to future workers, paratypes and vouchers that are illustrated and/or described herein are listed separately as "representatives," and alcohol-preserved materials for anatomical/biochemical study are listed separately (even though USNM does not assign them separate catalog numbers from dry materials of the same lots). In lot descriptions, "ad" refers to adult(s), "juv" to juveniles(s).

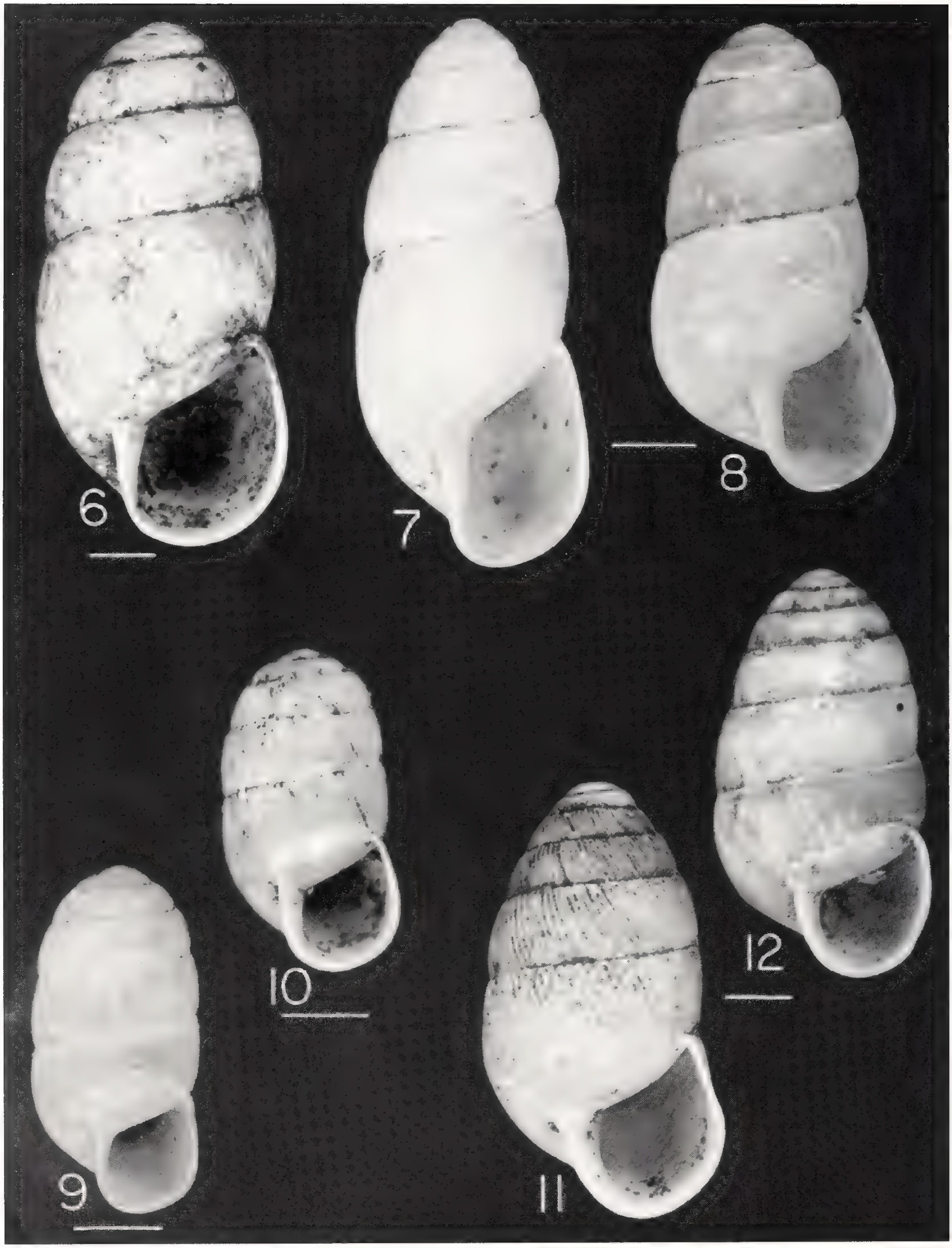
Class GASTROPODA
Subclass PULMONATA
Order STYLOMMATOPHORA
Superfamily STREPTAXOIDEA
Family STREPTAXIDAE Gray, 1860
Genus *Edentulina* Pfeiffer, 1856

Edentulina ambongoaboae sp. nov.
Fig. 6

HOLOTYPE. USNM 880370 (ex MBI 407.02DH, 1 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo, 290 m: dry deciduous forest; 26 Aug. 1995.

DRY PARATYPES. AMS C.203515 (ex MBI 407.02DP, 1 ad): type locality. MNHN (ex MBI 405.01DP, 1 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo, 320 m: baobab deciduous forest; 25 Aug. 1995. ANSP 401997 (ex MBI 405.01DP, 1 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo, 320 m: baobab deciduous forest; 25 Aug. 1995. USNM 880441-880443 (ex MBI 404-407DP, 3 lots; total 5 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo; Aug. 1995.

DESCRIPTION OF HOLOTYPE (a weathered shell). Shell elongate-ovoid, the aperture protruding slightly outside the ovoid profile. Height 23.8 mm, diameter 11.0 mm, whorl count 6.5, coiling tightness (whorls/ln height) 2.05. Body-whorl periphery gently rounded; suture moderately impressed, simple. Umbilicus a very narrow crevice, over half masked by reflected columellar peristome; umbilicus maximum diameter 0.5 mm. Aperture shape broad upright oval. Apertural lip reflected throughout, narrow at upper suture, then widening to a moderate reflection at the columellar insertion, with a narrow triangular, sloping shelf inside the columella. Aperture height 7.2 mm, width 6.7 mm. Preapertural deflection moderately upward, 0.1 whorl. Aperture side shape a reversed, very shallow comma.



Figs. 6-12. Fig. 6. *Edentulina ambongoaboae* sp. nov. holotype. Fig. 7. *E. ankaranae* sp. nov. holotype. Fig. 8. *Edentulina ambra* sp. nov. holotype. Figs. 9-10. *E. arenicola* (Morelet, 1860): Fig. 9 southeast of Diego Suarez, Fig. 10 Cap d'Ambre. Fig. 11. *E. rugosa* sp. nov. holotype. Fig. 12. *E. analamerae* sp. nov. holotype. Scale bars 3 mm.

Embryonic whorl count 2.6; diameter of first 1.5 whorls 2.3 mm. Embryonic sculpture smooth. Post-embryonic sculpture smooth, with faint traces of growth lines.

VARIATION. All adult specimens are remarkably uniform in size and shape.

ETYMOLOGY. For Mount Ambongoabo, the sole known locality for this species.

Edentulina ambra sp. nov.

Fig. 8

HOLOTYPE. USNM 880371 (ex MBI 191.01DH, 1 ad): 12°35'S, 49°9'E: Madagascar: Montagne d'Ambre National Park, 1260 m: rainforest; 11 July 1995.

DRY PARATYPES. AMS C.203516 (ex MBI 193.03DP, 1 juv): 12°34'S, 49°9'E: Madagascar: Montagne d'Ambre National Park, 1305 m: rainforest; 12 July 1995. MNHN (ex MBI 193.03DP, 1 juv): 12°34'S, 49°9'E: Madagascar: Montagne d'Ambre National Park, 1305 m: rainforest; 12 July 1995. USNM 880444-880456 (ex MBI 169-193DP, 8 lots; total 2 ad, 20 juv): 12°S, 49°E: Madagascar: Montagne d'Ambre National Park; July 1995.

ALCOHOL PARATYPES. USNM 880446-880457 (ex MBI 172-194AP, 10 lots; total 8 ad, 30 juv): 12°S, 49°E: Madagascar: Montagne d'Ambre National Park; July 1995.

DESCRIPTION OF HOLOTYPE. Shell a tapered column with bluntly fusiform apex, aperture scarcely protruding outside the shell's tapered-columnar profile. Height 16.6 mm, diameter 8.5 mm, whorl count 6.5, coiling tightness (whorls/ln height) 2.31. Body-whorl periphery gently rounded; suture moderately impressed, weakly crenulate. Umbilicus a circular well, fairly evenly expanding; umbilicus maximum diameter 1.1 mm. Aperture shape broad oval. Apertural lip scarcely reflected at the upper suture, broadening slightly below, then widening at the columella, where it grades into a wide, triangular, inward sloping shelf. Aperture height 4.6 mm, width 4.4 mm. No preapertural deflection. Aperture side shape nearly straight, but gently arching backward. Embryonic whorl count 2.5; diameter of first 1.5 whorls 2.1 mm. Embryonic sculpture low, narrowish, moderately spaced riblets. Post-embryonic sculpture weak ribs, nearly effaced below the periphery, but stronger at the umbilicus. Coloration pale straw-yellow.

VARIATION. Some specimens have a narrower aperture, with a height/width of up to 1.5.

ETYMOLOGY. For Montagne d'Ambre National Park.

Edentulina analamerae sp. nov.

Fig. 12

HOLOTYPE. USNM 880372 (ex MBI 210.02DH, 1 ad): 12°44'S, 49°29'E: Madagascar: Analamera Reserve, 35 m: dry deciduous floodplain; 16 July 1995.

DRY PARATYPES. AMS C.203517 (ex MBI 201.02DP, 1 ad): 12°44'S, 49°30'E: Madagascar: Analamera Reserve, 315 m: dry deciduous forest; 15 July 1995. USNM 880458 (ex MBI 201.02DP, 1 juv): 12°44'S, 49°30'E: Madagascar: Analamera Reserve, 315 m: dry deciduous forest; 15 July 1995.

DESCRIPTION OF HOLOTYPE (apex somewhat eroded). Shell fusiform-oval, aperture protruding only slightly outside the fusiform-oval profile. Height 18.8 mm, diameter 10.1 mm, whorl count 8.1, coiling tightness (whorls/ln height) 2.76. Body-whorl periphery gently rounded; body-whorl shoulder extremely narrow, sloped about 45 degrees; suture moderately impressed, weakly crenulate. Umbilicus a nearly circular, narrow well, widened by and half covered by rapid expansion in the final 0.2 whorl; umbilicus maximum diameter 1.8 mm. Aperture obliquely egg-shaped. Apertural lip reflected throughout, narrowly at the upper suture, moderately thereafter, widening at the columella, with a narrow, steeply sloping triangular shelf below the columellar peristome. Aperture height 5.0 mm, width 5.3 mm. Preapertural deflection gradually upward, 0.3 whorl. Aperture side shape nearly straight, but slightly arching bacward. Embryonic whorl count 2.8; diameter of first 1.5 whorls 2.0 mm. Embryonic sculpture smooth. Post-embryonic sculpture smoothish, with low, faint ribs. Coloration beigish white.

ETYMOLOGY. For Analamera Reserve.

Edentulina ankaranae sp. nov.

Fig. 7

HOLOTYPE. USNM 880373 (ex MBI 580.01DH, 1 ad): 12°58'S, 49°5'E: Madagascar: Ankarana Reserve, 95 m: dry deciduous forest; 26 Aug. 1995.

DRY PARATYPES. AMS C.203518-C.203522 (ex MBI 802-815DP; 5 lots; total 9 ad, 14 juv): Madagascar: Ankarana Reserve; Oct. 1994. MNHN (ex MBI 564.02DP, 1 ad): 12°55'S, 49°5'E: Madagascar: Ankarana Reserve, 95 m: dry deciduous forest; 22 Aug. 1995. ANSP 401998 (ex MBI 564.02DP, 1 ad): 12°55'S, 49°5'E: Madagascar: Ankarana Reserve, 95 m: dry deciduous forest; 22 Aug. 1995. USNM 880459-880472 (ex MBI 557-580DP, 802-815DP, 14 lots; total 23 ad, 54 juv): Madagascar: Ankarana Reserve, 90 m: dry deciduous forest; 1994, 1995.

DESCRIPTION OF HOLOTYPE. Shell fusiform, aperture protruding slightly outside the fusiform profile. Height 19.5 mm, diameter 8.0 mm, whorl count 6.4, coiling tightness (whorls/ln height) 2.15. Body-whorl periphery very gently rounded; suture mildly impressed, simple. Umbilicus a narrow well, partially masked by reflected columellar peristome; umbilicus maximum diameter 0.5 mm. Aperture shape auriculate-oval. Apertural lip thinly reflected along outer margin, funneled basally, broadly reflected along columella and broadening greatly to the columellar insertion. Aperture height 6.1 mm, width 4.3 mm. Preapertural deflection gradually, then--abruptly--moderately upward, 0.3 whorl. Aperture side shape a reversed very shallow comma, but straight and just slightly recurved below. Embryonic whorl count 2.3; diameter of first 1.5 whorls 1.9 mm. Embryonic sculpture smooth. Post-embryonic sculpture smooth, with faint growth lines and subsutural traces of ribs. Coloration ivory.

VARIATION. Adult whorls range 5.9-6.9, height 13.6-22.5 mm, coiling tightness (whorls/ln height) 2.1-2.3; shell height/diameter 2.1-2.4; embryonic whorls 2.3-2.5; diameter of first 1.5 whorls 1.7-1.9 mm.

ETYMOLOGY. For Ankarana Reserve.

Edentulina antankarana sp. nov.

Fig. 13

HOLOTYPE. USNM 880374 (ex MBI 810.02DH, 1 broken ad): 12°54'S, 49°6'E: Madagascar: Ankarana Reserve, 90 m; 10 Oct. 1994.

DRY PARATYPES. AMS C.203523 (ex MBI 810.02DP, 2 juv): type locality: 12°54'S, 49°6'E: Madagascar: Ankarana Reserve, 90 m; 10 Oct. 1994. USNM 880473 (ex MBI 810.02DP, 2 juv): type locality.

DESCRIPTION OF HOLOTYPE (adult shell from which the final 0.5 whorls are broken off, leaving some traces of adult morphology). Shell fusiform-oval. Height 22.0 mm, diameter 10.8 mm, whorl count 7.8, coiling tightness (whorls/ln height) 2.54. Body-whorl periphery gently rounded; suture well impressed, faintly crenulate. Umbilicus a narrow well with tear-drop-shaped periphery, widening abruptly (judging from scars on shell). Aperture shape uncertain, but elongate. Aperture height 6.0 mm, width 4.6 mm. Preapertural deflection weakly upward, 0.2 whorl (judging from scars on shell). Embryonic whorl count 2.8; diameter of first 1.5 whorls 1.9 mm. Embryonic sculpture very faint riblets. Post-embryonic sculpture strong, low, moderately wide, irregularly spaced ribs.

ETYMOLOGY. For the tribe of people (Antankarana) living near Ankarana Reserve.

Edentulina arenicola (Morelet, 1860)

Figs. 9, 10

REPRESENTATIVES. USNM 894278 (Fig. 9, ex MBI 217.03DR, 1 ad): 12°23'S, 49°19'E: Madagascar: Montagne des Orchides, 360 m: dry deciduous forest; 20 July 1995. USNM 894279 (Fig. 10, ex MBI 400.07DR, 1 ad): 12°10'S, 49°13'E: Madagascar: Cap d'Ambre, la Butte Bobaomby, 70 m: dry deciduous forest; 24 Aug. 1995.

OTHER DRY VOUCHERS. AMS C.203556 (ex MBI 217.03D, 1 ad): 12°23'S, 49°19'E: Madagascar: Montagne des Orchides, 360 m: dry deciduous forest; 20 July 1995. AMS C.203548 (ex MBI 401.01D, 3 ad): 12°11'S, 49°13'E: Madagascar: Cap d'Ambre, la Butte Bobaomby, 205 m: dry deciduous-baobab forest; 24 Aug. 1995. MNHN (ex MBI 217.03D, 1 ad): 12°23'S, 49°19'E: Madagascar: Montagne des Orchides, 360 m: dry deciduous forest; 20 July 1995. MNHN (ex MBI 401.01D, 1 ad): 12°11'S, 49°13'E: Madagascar: Cap d'Ambre, la Butte Bobaomby, 205 m: dry deciduous-baobab forest; 24 Aug. 1995. ANSP 401999 (ex MBI 217.03D, 1 ad): 12°23'S, 49°19'E: Madagascar: Montagne des Orchides, 360 m: dry deciduous forest; 20 July 1995. ANSP 402000 (ex MBI 401.01D, 1 ad): 12°11'S, 49°13'E: Madagascar: Cap d'Ambre, la Butte Bobaomby, 205 m: dry deciduous-baobab forest; 24 Aug. 1995. USNM 880474-880478 (ex MBI 215-219D, 4 lots; total 10 ad, 31 juv): Montagne des Orchides; 20 July 1995. USNM 880479 (ex MBI 222.02D, 10 ad, 22 juv): 12°19'S, 49°20'E: Madagascar: Montagne des Francais, 230 m: dry deciduous forest; 21 July 1995. USNM 880480 (ex MBI 401.01D, 52 ad, 103 juv): 12°11'S, 49°13'E: Madagascar: Cap d'Ambre, la Butte Bobaomby, 205 m: dry deciduous-baobab forest; 24 Aug. 1995.

ALCOHOL VOUCHER. USNM 880477 (ex MBI 218.02A, 1 juv): 12°23'S, 49°19'E: Madagascar: Montagne des Orchides, 385 m: dry deciduous forest; 20 July 1995.

DESCRIPTION. Shell barrel-shaped, with aperture not protruding to conspicuously protruding outside the shell profile. Height 9.6-13.8 mm, height/diameter 1.7-2.0, whorl count 6.3-7.5, coiling tightness (whorls/ln height) 2.7-3.0. Body-whorl periphery flattened; suture moderately impressed, with a crenulate appearance due to rib sculpture. Umbilicus initial shape ranging from a tiny, round pinhole to a narrow well with tear-drop-shaped periphery; with abrupt widening in the final 0.2 whorls, in which the body whorl masks the preceding umbilicus; umbilicus maximum diameter approximately 0.9 mm. Aperture shape squarish-ovate to very broadly auriculate. Apertural lip reflected throughout, narrow at the upper suture, broad at the columella. Aperture size ranges from fairly small and narrow (Fig. 9; one extreme, aberrant specimen--not illustrated--with an arched columella has an aperture height/width of 2.0) to large and broad (Fig. 10; aperture height/width approaching 1.0). Preapertural deflection weakly to moderately upward, <0.05 to 0.1 whorl. Aperture side shape a reversed, very shallow comma. Embryonic whorl count 1.9-2.2; diameter of first 1.5 whorls 1.2-1.5 mm. Embryonic sculpture smooth, sometimes with a faint trace of close-set spiral striae detectable at 40x magnification. Post-embryonic sculpture consisting of strong, broad ribs. Color ivory.

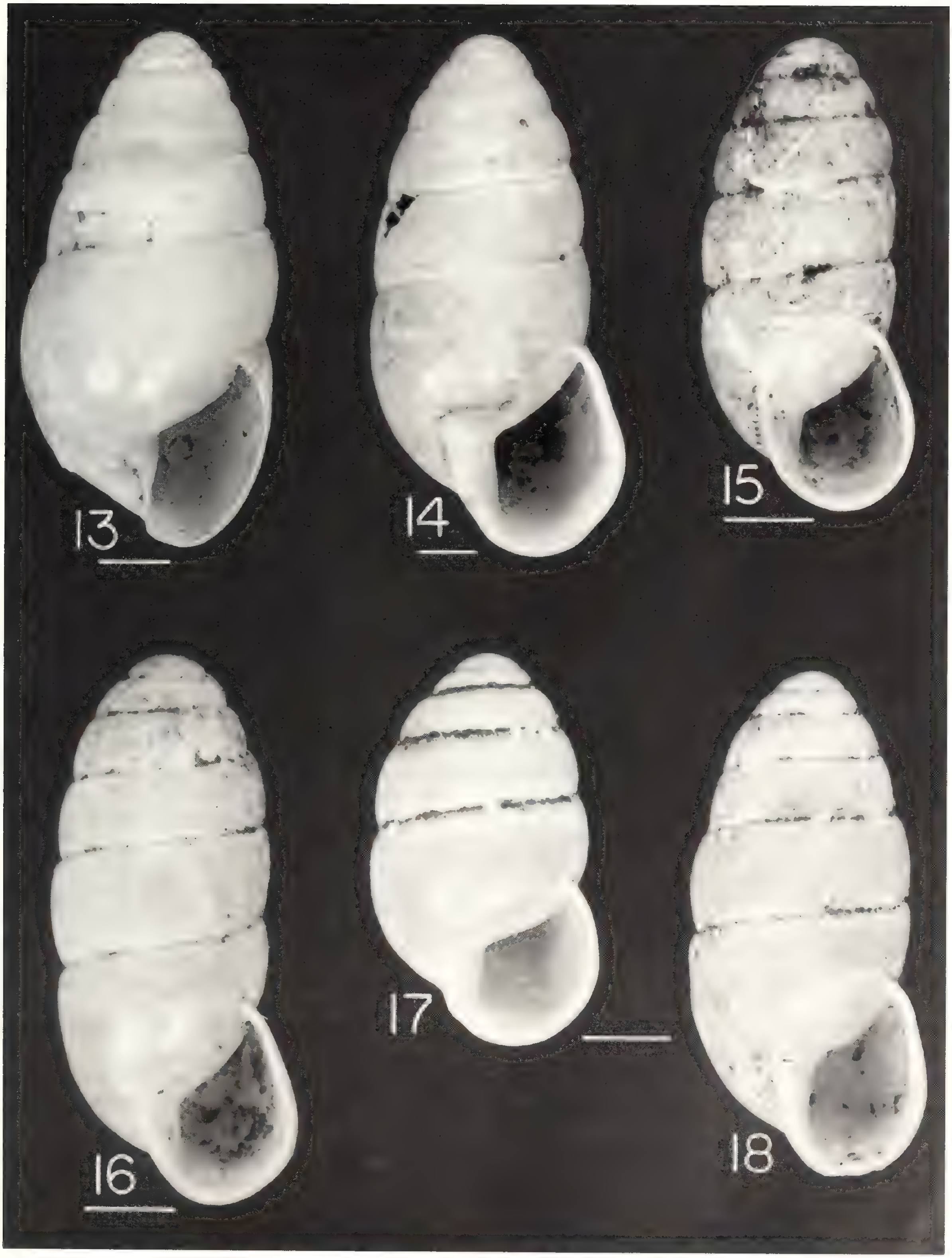
COMMENTS. Disposition of the type is unknown. The type locality is Port Leven, east of the two widely separated populations represented in Figs. 9 and 10, this paper. Previously published illustrations of *E. arenicola* are poor and misleading.

Edentulina battistinii Fischer-Piette, F. Blanc, and Salvat, 1975

Fig. 14

REPRESENTATIVE. USNM 894280 (ex MBI 245.03DR, 1 ad): 19°8'S, 44°48'E: Madagascar: S Bemaraha Reserve: dry forest; 14 June 1995.

OTHER DRY VOUCHERS. AMS C.203524, C.203525, C.203527-C.203534 (ex MBI 245-254D, 484-494D; 10 lots; total 38 ad, 18 juv): Madagascar: Bemaraha Reserve; 1995, 1996. MNHN (ex MBI 245.03D, 1 ad): 19°8'S, 44°48'E: Madagascar: S Bemaraha Reserve: dry forest; 14 June 1995. ANSP 402001 (ex



Figs. 13-18. Fig. 13. *Edentulina antankarana* sp. nov. holotype. Fig. 14. *E. battistinii* Fischer-Piette, F. Blanc, and Salvat, 1975, Bemaraha Reserve. Figs. 15-18. *E. bemarahae* sp. nov.: Fig. 16 holotype; Figs. 15, 17, 18 paratypes. Scale bars 3 mm.

MBI 254.02D, 1 ad): 19°8'S, 44°52'E: Madagascar: S Bemaraha Reserve, 100 m: forest; 18 June 1995. USNM 880481-880492 (ex MBI 245-254D, 483-494D, 11 lots; total 40 ad, 17 juv): Madagascar: Bemaraha Reserve; 1995, 1996.

ALCOHOL VOUCHER. USNM 880492 (ex MBI 494.08A, 1 juv): 18°45'S, 44°45'E: Madagascar: N Bemaraha Reserve, 280 m: semideciduous forest; 29 June 1996.

DESCRIPTION. Shell bluntly fusiform, aperture protruding greatly outside the fusiform profile. Height 17.9-33.5 mm, height/diameter 1.8-2.5 mm, whorl count 6.8-8.8, coiling tightness (whorls/ln height) 2.4-2.5. Body-whorl periphery gently rounded, slightly flattened; body-whorl shoulder extremely narrow, sloped about 60 degrees; suture moderately impressed, very faintly crenulate. Umbilicus a moderately narrow well with tear-drop-shaped periphery, with abrupt widening in final 0.3 whorl. Aperture shape very broad auriculate-oval. Apertural lip thickly reflected, from narrow at the upper suture, through moderate and somewhat funneled at the base, to broad at the columella, with a triangular, gently sloping shelf inside the columella. Preapertural deflection gradually, then--abruptly--moderately upward, approximately 0.3 whorl. Aperture side shape a broad, distorted S, the upper curve long, the bottom curve short, both curves very shallow. Embryonic whorl count 2.9-3.0; diameter of first 1.5 whorls 2.2-2.4 mm. Embryonic sculpture faint riblets. Post-embryonic sculpture consists of strong, somewhat thin, moderately spaced ribs.

Edentulina bemarahae sp. nov.

Figs. 15, 16, 17, 18

HOLOTYPE. USNM 880375 (Fig. 16, ex MBI 247.01DH, 1 ad): 19°8'S, 44°52'E: Madagascar: S Bemaraha Reserve: riverine gallery forest; 15 June 1995.

DRY PARATYPE REPRESENTATIVES. USNM 880376 (Fig. 17, ex MBI 247.01DPR, 1 ad): type locality. USNM 880377 (Fig. 15, ex MBI 245.02DPR, 1 ad): 19°8'S, 44°o48'E: Madagascar: S Bemaraha Reserve: dry forest; 14 June 1995. USNM 880378 (Fig. 18, ex MBI 254.01DPR, 1 ad): 19°2'S, 44°48'E: Madagascar: S Bemaraha Reserve: forest.

OTHER DRY PARATYPES. AMS C.203535-C.203547 (ex MBI 245-255DP, 483-495DP; 13 lots; total 24 ad, 28 juv): Madagascar: Bemaraha Reserve; 1995, 1996. MNHN (ex MBI 247.01DP, 1 ad): type locality. ANSP 402002 (ex MBI 247.01DP, 1 ad): type locality. USNM 880493-880507 (ex MBI 245-255DP, 483-495DP, 14 lots; total 25 ad, 31 juv): Bemaraha Reserve; 1995, 1996.

ALCOHOL PARATYPES. USNM 880497 (ex MBI 250.01AP, 1 juv): 19°8'S, 44°49'E: Madagascar: S Bemaraha Reserve; 16 June 1995. USNM 880503 (ex MBI 489.02AP, 2 juv): 18°41'S, 44°43'E: Madagascar: N Bemaraha Reserve, 150 m: semideciduous forest; 27 June 1996.

DESCRIPTION OF HOLOTYPE. Shell bluntly fusiform, aperture protruding greatly outside the fusiform profile. Height 18.2 mm, diameter 8.0 mm, whorl count 7.9, coiling tightness (whorls/ln height) 2.72. Body-whorl periphery gently rounded; body-whorl shoulder very narrow, sloped about 30 degrees; suture moderately impressed, simple to weakly crenulate. Umbilicus a narrow well with tear-drop-shaped periphery, with abrupt widening in final 0.1 whorl; umbilicus maximum diameter 0.8 mm. Aperture shape broadly auriculate. Apertural lip thickly reflected, narrow at upper suture, broad at columella with sloping triangular shelf below. Aperture height 4.5 mm, width 4.3 mm. Preapertural deflection gradually, then—briefly—strongly upward, 0.5 whorl. Aperture side shape reversed very shallow comma. Embryonic whorl count 2.7; diameter of first 1.5 whorls 1.9 mm. Embryonic sculpture broad, close-set riblets. Post-embryonic sculpture strong, moderately wide, moderately spaced ribs. Coloration ivory.

VARIATION (Figs. 15-18). Adult whorls range 6.5-7.9, height 13.5-18.2, coiling tightness (whorls/ln height) 2.5-2.8; shell height/diameter 1.8-2.3; umbilicus/shell diameter 0.1-0.2; aperture height/width 1.0-1.2; aperture width/shell diameter 0.5-0.6; pre-apertural deflection 0.2-0.5 whorl; embryonic whorls 2.7-2.9; diameter of first 1.5 whorls 1.6-1.9; post-embryonic sculptural ribs vary considerably in width and density.

ETYMOLOGY. For Bemaraha Reserve.

Edentulina florensi sp. nov.

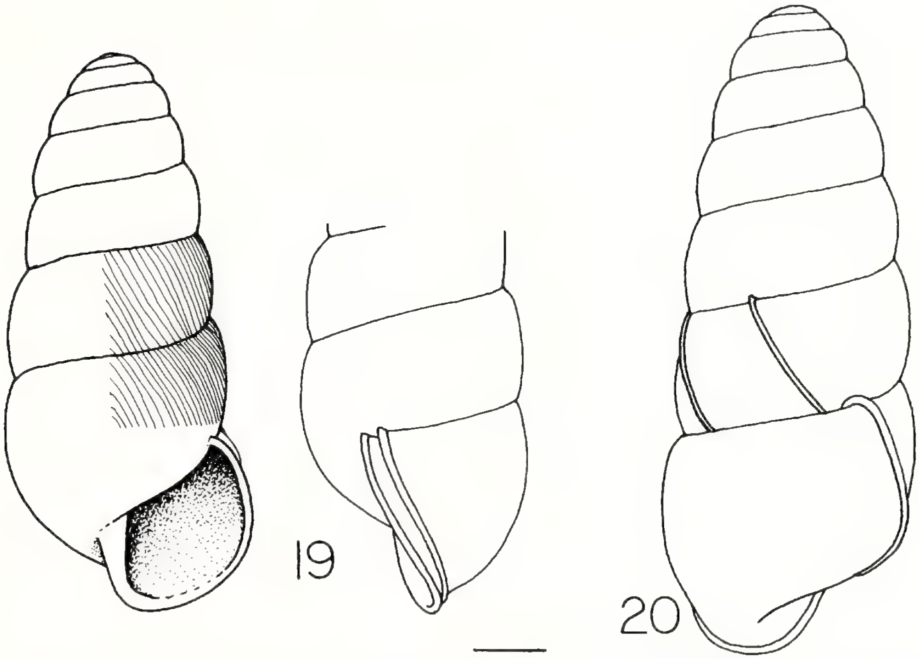
Figs. 19, 20

HOLOTYPE. USNM 880379 (Fig. 19, ex MBI 490.41DH, 1 ad): 18°45'S, 44°45'E: Madagascar: N Bemaraha Reserve, 280 m: semideciduous forest. 27-Jun-96.

DRY PARATYPE REPRESENTATIVE. USNM 880380 (Fig. 20, ex MBI 488.41DP, 1 ad): 18°47'S, 44°47'E: Madagascar: N Bemaraha Reserve, 250 m: riverine scrub. 27-Jun-96.

OTHER DRY PARATYPES. AMS C.203549-C.203554 (ex MBI 484-494DP; 6 lots; total 47 ad, 39 juv): 18°3'S, 44°31'E: Madagascar: N Bemaraha Reserve, 250 m: dry deciduous forest. 23-Jun-96. MNHN (ex MBI 494.41DP, 2 ad): 18°45'S, 44°45'E: Madagascar: N Bemaraha Reserve, 280 m: semideciduous forest. 29-Jun-96. ANSP 402003 (ex MBI 494.41DP, 2 ad): 18°45'S, 44°45'E: Madagascar: N Bemaraha Reserve, 280 m: semideciduous forest. 29-Jun-96. USNM 880508-880514 (ex MBI 484-495DP, 8 lots; total 44 ad, 42 juv): Madagascar: N Bemaraha Reserve. Jun-96.

DESCRIPTION OF HOLOTYPE. Shell tall and bluntly pyramidal. Height 22.8 mm, diameter 9.2 mm, whorl count 8.2, coiling tightness (whorls/ln height) 2.62. Body-whorl periphery gently rounded, slightly flattened; body-whorl shoulder narrow, sloped about 60 degrees; suture moderately impressed, simple to weakly crenulate. Umbilicus a narrow well with tear-drop-shaped periphery, about half covered by the reflected columellar peristome; umbilicus maximum diameter 0.8 mm. Aperture shape auriculate-oval. Apertural lip very narrowly reflected at the upper suture and to the basal area, whence it expands to a broadly triangular columellar insertion, within which is a narrow, trian-



Figs. 19-20. *Edentulina florensi* sp. nov.: Fig. 19 holotype in two views; Fig. 20 paratype. Scale bar 3 mm.

gular, steeply sloping shelf. Aperture height 5.0 mm, width 5.1 mm. Preapertural deflection gradually upward, 0.2 whorl. Aperture side shape a reversed, very shallow comma. Embryonic whorl count 2.8; diameter of first 1.5 whorls 2.2 mm. Embryonic sculpture riblets. Post-embryonic sculpture moderately strong, thin ribs, moderately spaced; a conspicuous, pre-apertural, duplicate peristome is present. Coloration ivory.

VARIATION (Figs. 19-20, in part). Presumed adult whorls range 6.7-9.2, height 15.8-29.4; shell height/diameter 1.9-3.0; coiling tightness (whorls/ln height) 2.4-2.7; one or more pre-apertural, duplicate peristomes occur in about three-fourths of presumed adults.

ETYMOLOGY. For Vincent Florens, in recognition of his outstanding contribution to the first Bemaraha expedition.

Edentulina minor (Morelet, 1851)

Figs. 1, 2, 3, 4

NEW SYNONYMS. *Edentulina alluaudi* (Dautzenberg, 1895) (Fig. 3; Fischer-Piette *et al.*, 1994:57, plate IV fig. 11). The slender shape of this variant from Montagne d'Ambre (see below) falls within the morphological range of *E. minor* from other sites such as Namoroka Reserve (see below).

Edentulina gaillardi Fischer-Piette and Bedoucha, 1964 (Fig. 4; Fischer-Piette *et al.*, 1994:57, plate IV fig. 11). A wide-apertured, strong-ribbed variant of *E. minor* from Ankarafantsika Reserve and environs (including Ampijoroa Reserve, see below), which also occurs within *E. minor's* range of variation within Namoroka Reserve (see below).

Edentulina intermedia (Morelet, 1851) (Fischer-Piette *et al.*, 1994:50, fig. 33). Fischer-Piette was unable to locate the holotype, so he reproduced Morelet's inadequate figure and measurements; he also commissioned numerous collections at the sole known locality (Port-Léven), but without success (Fischer-Piette and Bedoucha, 1964). Fortunately, however, Tryon (1885:83; plate 17, fig. 20) had adequately illustrated and remeasured the holotype, which falls within *E. minor's* known range of variation.

Edentulina montis Fischer-Piette, F. Blanc, and Salvat, 1975 (Fischer-Piette *et al.*, 1994: 57, plate IV fig. 12-14). Despite its narrow umbilicus, this species seems to fall within the wide variation of *E. minor*, which occurs at the type locality of *E. montis*, Montagne des Francais (see below).

Edentulina stumpfii Kobelt, 1904 (Fischer-Piette *et al.*, 1994: 51, Fig. 34). On Nosy Be, the type locality of *E. stumpfii*, many *E. minor* were collected (see below), within whose range of variation *E. stumpfii* seems to fall.

REPRESENTATIVES. USNM 894281 (Figs. 1 and 2, ex MBI 203.03DR, 2 ad): 12°44'S, 49°30'E: Madagascar: Analamera Reserve, 285 m: bamboo-dry deciduous thicket; 16 July 1995. USNM 894282 (Fig. 3, ex MBI 184.01DR, 1 ad): 12°36'S, 49°9'E: Madagascar: Montagne d'Ambre National Park, 1165 m: rainforest; 10 July 1995. USNM

894283 (Fig. 4, ex MBI 61.03DR, 1 ad): 16°23'S, 45°18'E: Madagascar: Namoroka Reserve, 105 m: dry deciduous forest; 25 May 1995.

OTHER DRY VOUCHERS. AMS C.203514 (ex MBI 184.01D, 1 ad): 12°36'S, 49°9'E: Madagascar: Montagne d'Ambre National Park, 1165 m: rainforest; 10 July 1995. AMS C.203555 (ex MBI 83.01D, 1 ad): 16°17'S, 46°49'E: Madagascar: Ampijoroa Reserve, 95 m: hardwood deciduous forest; 3 June 1995. AMS C.203558 (ex MBI 55.03D, 2 ad): 16°23'S, 45°21'E: Madagascar: Namoroka Reserve, 110 m: dry deciduous forest; 21 May 1995. AMS C.203559 (ex MBI 487.01D, 3 ad, 4 juv): 18°'S, 44°'E: Madagascar: N Bemaraha Reserve; 25 June 1996. AMS C.203560 (ex MBI 805.03D, 6 ad, 8 juv): 13°1'S, 49°0'E: Madagascar: Ankarana Reserve, 50 m; 8 Oct. 1994. MNHN (ex MBI 55.03D, 2 ad): 16°23'S, 45°21'E: Madagascar: Namoroka Reserve, 110 m: dry deciduous forest; 21 May 1995. MNHN (ex MBI 83.01D, 1 ad): 16°17'S, 46°49'E: Madagascar: Ampijoroa Reserve, 95 m: hardwood deciduous forest; 3 June 1995. MNHN (ex MBI 184.01D, 1 ad): 12°36'S, 49°9'E: Madagascar: Montagne d'Ambre National Park, 1165 m: rainforest; 10 July 1995. ANSP 402004 (ex MBI 55.03D, 2 ad): 16°23'S, 45°21'E: Madagascar: Namoroka Reserve, 110 m: dry deciduous forest; 21 May 1995. ANSP 402005 (ex MBI 83.01D, 1 ad): 16°17'S, 46°49'E: Madagascar: Ampijoroa Reserve, 95 m: hardwood deciduous forest; 3 June 1995. ANSP 402006 (ex MBI 184.01D, 1 ad): 12°36'S, 49°9'E: Madagascar: Montagne d'Ambre National Park, 1165 m: rainforest; 10 July 1995. USNM 880515-880527 (ex MBI 55-74D, 13 lots; total 148 ad, 148 [no mistake] juv): Madagascar: Namoroka Reserve; May 1995. USNM 880528-880531 (ex MBI 81-84D, 4 lots; total 28 ad, 57 juv): 16°17'S, 46°49'E: Madagascar: Ampijoroa Reserve; June 1995. USNM 880532 (ex MBI 86.01D, 1 juv): 16°8'S, 47°0'E: Madagascar: Ankarafantsika Reserve, 160 m: dry deciduous forest; 5 June 1995. USNM 880533-880549 (ex MBI 118-150D, 17 lots; total 14 ad, 42 juv): 13°S, 48°E: Madagascar: Nosy Be: Lokobe Reserve; June 1995. USNM 880550 (ex MBI 168.01D, 2 ad, 1 juv): 13°34'S, 48°45'E: Madagascar: Galoko Escarpment, 225 m: hardwood-palm rainforest; 4 July 1995. USNM 880551-880560 (ex MBI 177-194D, 10 lots; total 6 ad, 19 juv): 12°S, 49°E: Madagascar: Montagne d'Ambre National Park; July 1995. USNM 880561-880575 (ex MBI 199-214D, 15 lots; total 39 ad, 102 juv): 12°S, 49°E: Madagascar: Analamera Reserve; July 1995. USNM 880576-880577 (ex MBI 217-219D, 2 lots; total 6 ad, 7 juv): 12°23'S, 49°19'E: Madagascar: Montagne des Orchides; 20 July 1995. USNM 880578-880579 (ex MBI 220-221D, 2 lots; total 1 ad, 10 juv): 12°19'S, 49°20'E: Madagascar: Montagne des Francais; 21 July 1995. USNM 880580-880591 (ex MBI 230-240D, 402-407D, 12 lots; total 25 ad, 80 juv): Madagascar: Cap d'Ambre; 1995. USNM 880592 (ex MBI 410.01D, 1 juv): 12°26'S, 49°12'E: Madagascar: W of Sakaramy, S of Diego Suarez, 470 m: dry deciduous viny forest; 26 Aug. 1995. USNM 880593-880600 (ex MBI 412-421D, 8 lots; total 10 ad, 29 juv): Madagascar: Andavakoera massif, N of Betsiaka; 1995. USNM 880601 (ex MBI 487.01D, 4 ad, 4 juv): 18°'S, 44°'E: Madagascar: N Bemaraha Reserve; 25 June 1996. USNM 880602 (ex MBI 548.01D, 9 juv): 13°28'S, 48°21'E: Madagascar: Nosy Komba, 622 m: cleared, former forest; 23 June 1995. USNM 880603-880605 (ex MBI 564-572D, 805D, 3 lots; total 9 ad, 13 juv): Madagascar: Ankarana Reserve; 1994, 1995.

ALCOHOL VOUCHERS. USNM 880516-880523 (ex MBI 56-70A, 2 lots; total 2 juv): 16°S, 45°E: Madagascar: Namoroka Reserve; May 1995. USNM 880530-880531 (ex MBI 83-84A, 2 lots; total 2 juv): 16°17'S, 46°49'E: Madagascar: Ampijoroa Reserve; June 1995. USNM 880538-880549 (ex MBI 125-150A, 7 lots; total 11 juv): Madagascar: Nosy Be: Lokobe Reserve; June 1995. USNM 880567-880574 (ex MBI 206-213A, 2 lots; total 1 ad, 2 juv): 12°S, 49°E: Madagascar: Analamera Reserve; July 1995. USNM 880585 (ex MBI 239.01A, 1 ad): 12°0'S, 49°17'E: Madagascar: Cap d'Ambre, near Ambatojanahary, 40 m: dry deciduous forest; 25 July 1995. USNM 880594-880600 (ex MBI 413-421A, 3 lots; total 3 juv): 13°S, 49°E: Madagascar: Andavakoera massif, N of Betsiaka; 1995.

DESCRIPTION. Shell acute-oval to fusiform, aperture protruding moderately to greatly outside the oval-to-fusiform profile. Height 16.4-37.2 mm, height/diameter 1.6-2.0, whorl count 6.9-9.0, coiling tightness (whorls/ln height) 2.4-2.5. Body-whorl periphery rounded; body-whorl shoulder a very narrow shelf defined by a subsutural cord; suture shallowly to moderately impressed, simple to faintly undulating to faintly crenulate. Subsutural spiral cord present. Umbilicus a slit-like crevice or crease to a tear-drop-shaped well, abruptly widened by rapid expansion in final 0.2-0.4 whorl; umbilicus maximum diameter/shell diameter 0.2. Aperture shape broad auriculate-oval. Apertural lip unreflected to narrowly reflected at the upper suture, moderately and thickly relected thereafter, the columella broadened by a triangular shelf slanting inward within the aperture. Aperture width/shell diameter 0.5-0.6; apertural height/width 1.0-2.0. Preapertural deflection weakly to moderately upward, 0.1-0.2 whorl. Aperture side shape a reversed, shallow comma, but straight and slightly recurved below. Embryonic whorl count 2.6-3.1; diameter of first 1.5 whorls 1.8-2.0 mm. Embryonic sculpture smooth, sometimes with very faint traces of low riblets in the final half whorl. Post-embryonic sculpture weak to moderately strong, closely spaced ribs. Coloration ivory to yellowish beige.

Edentulina nitens (Dautzenberg, 1895)

Fig. 5

REPRESENTATIVE. USNM 894284 (ex MBI 210.04DR, 1 ad): 12°44'S, 49°29'E: Madagascar: Analamera Reserve, 35 m: dry deciduous floodplain; 16 July 1995.

DESCRIPTION OF REPRESENTATIVE (embryonic shell severely fractured and repaired during life). Shell blunt elongate-oval. Height 20.6 mm, diameter 10.4 mm, whorl count 6.7, coiling tightness (whorls/ln height) 2.21. Body-whorl periphery flattened; suture well impressed, simple. Umbilicus a narrow well, scarcely widened in final 0.1 whorl; umbilicus maximum diameter 0.8 mm. Aperture shape broadly auriculate-oval. Apertural lip nearly unreflected at the upper suture, then narrow, widening greatly at the columella into a triangular insertion, with a narrow, steep shelf below the columella. Aperture height 6.1 mm, width 5.3 mm. No preapertural deflection. Aperture side shape a reversed, very shallow comma, but straight and just slightly recurved below. Embryonic whorl count 2.4; diameter of first 1.5 whorls 1.8 mm. Embryonic sculpture smooth. Post-embryonic sculpture smooth, with faint, very regularly spaced growth lines. Coloration ivory.

Edentulina rugosa sp. nov.

Fig. 11

HOLOTYPE. USNM 880381 (ex MBI 407.04DH, 1 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo, 290 m: dry deciduous forest; 26 Aug. 1995.

DRY PARATYPES. AMS C.203561 (ex MBI 406.01DP, 1 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo, 310 m: baobab deciduous forest; 25 Aug. 1995. MNHN (ex MBI 405.03DP, 1 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo, 320 m: baobab deciduous forest; 25 Aug. 1995. ANSP 402007 (ex MBI 405.03DP, 1 ad): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo, 320 m: baobab deciduous forest; 25 Aug. 1995. USNM 880606-880607 (ex MBI 405-407DP, 2 lots; total 4 juv): 12°15'S, 49°15'E: Madagascar: Cap d'Ambre, Ambongoabo; Aug. 1995. USNM 880608-880609 (ex MBI 410-411DP, 2 lots; total 1 ad, 5 juv): 12°26'S, 49°12'E: Madagascar: W of Sakaramy, S of Diego Suarez; Aug. 1995.

DESCRIPTION OF HOLOTYPE. Shell fusiform-oval. Height 20.2 mm, diameter 10.7 mm, whorl count 7.4, coiling tightness (whorls/ln height) 2.46. Body-whorl periphery slightly flattened; suture mildly impressed, strongly crenulate from ribbed sculpture. Umbilicus a moderately wide well, rapidly expanded in last 0.1 whorl; umbilicus maximum diameter 1.5 mm. Aperture shape broadly auriculate. Apertural lip narrowly reflected at the upper suture, broadening to widely reflected at the columella, which continues inward as a triangular sloping shelf. Aperture height 5.8 mm, width 5.8 mm. Preapertural deflection gradually upward, 0.2 whorl. Aperture side shape a reversed, extremely shallow comma, straightened and very slightly recurved at the bottom. Embryonic whorl count 2.6. Embryonic sculpture smooth, with a hint of dense spiral lines visible at 40x magnification. Post-embryonic sculpture very strongly ribbed. Coloration light beige.

VARIATION. Adult height ranges from 16.7 to 22.8 mm.

ETYMOLOGY. For the conspicuous sculpture that is strongly wrinkled (Latin "rugosa") or ribbed.

DISCUSSION

Fischer-Piette *et al.*'s (1994) faunal list tentatively included two extra-Madagascan *Edentulina: E. ovoidea* (Bruguiere, 1792) of East Africa and the Comores, and *E. dussumieri* (Dufo, 1840) of the Seychelles. Neither of those species was encountered in the 1992-1995 survey. *E. ovoidea* should remain tentatively on the faunal list, because it reportedly was introduced (misguidedly) as a biological control agent to Madagascar (Fischer-Piette *et al.*, 1994:49-50). It seems safe, however, to remove *E. dussumieri*, because it has never been collected in Madagascar since Morelet's old and non-vouchered report.

Based on collections made in 1992-1995 from at or near their type localities, four species are synonymized above under *E. minor* (Morelet, 1851): *E. alluaudi* (Dautzenberg, 1895); *E. gaillardi* Fischer-Piette and Bedoucha, 1964; *E. montis* Fischer-Piette, F. Blanc, and Salvat, 1975; and *E. stumpfii* Kobelt, 1904. *E. intermedia* (Morelet, 1851) is also synonymized under *E. minor*, based on Tryon's (1885) redescription of the holotype, and based on numerous barren collections at the type locality (Fischer-Piette and Bedoucha, 1964).

Thus, of Fischer-Piette *et al.*'s (1994) list of 13 native species of Madagascan *Edentulina*, this paper deletes one, transfers three, and synonymizes five. This leaves four, all of which were collected in 1992-1995 and are described (from representatives) above. With the addition of 7 new species, also described above, Madagascar's list of *Edentulina* now totals 11 native species and, possibly, the one introduced species.

Many promising regions of Madagascar remain uncollected, so exploration should yield additional new species of *Edentulina*.

ACKNOWLEDGMENTS

Funded by the U.S. National Science Foundation (DEB 9201060), with some additional funding provided by Owen Griffiths. Permits were issued by the Madagascar government agencies DEF and ANGAP. Ranomafana National Park Project gave logistic support. Many people collected or otherwise assisted, but particular thanks go to Owen Griffiths, who conducted both Bemaraha expeditions and an Ankarana expedition; Dr. Tim Pearce, who conducted the bulk of the Montagne d'Ambre and a second Ankarana expedition; and Felix Rakotomalala *(requiescat in pace)*, Tim Pearce, Jean Rakotoarison, and Roger Randalana, who were primary assistants in both the field and the sorting lab.

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AMERICAN MALACOLOGICAL SOCIETY
FINANCIAL REPORT

General Accounts

1998 Income and Expenses

TOTAL ASSETS (1/1/98)			$131,991.62
INCOME			$46,954.96
Membership Dues (1996, 1997, 1998)		12,818.00	
Membership Dues (1999)		1,515.00	
Interest and Dividends		3,966.35	
Money Market Account Interest	1,503.06		
Life Membership Endowment Fund	236.88		
Symposium Endowment Funds	2,226.41		
Publications Income		7,413.40	
AMB Foreign Subscriptions	1,696.00		
AMB Domestic Subscriptions	2,994.00		
AMB Page Charges	1,716.00		
AMB Reprint Charges	680.00		
Sales of Other Publications	327.40		
Annual Meetings		16,910.33	
1997 Meeting Surplus	97.13		
**1998 Meeting Surplus	12,279.50		
1997 Auction Proceeds	978.00		
1998 Auction Proceeds	3,555.70		
Donations		4,299.38	
Symposium Endowment Fund	440.00		
Student Grant Endowment Fund	939.00		
Miscellaneous Income		32.50	
EXPENSES			$24,731.48
Secretary Expenses		208.00	
Membership Chairman Expenses		133.15	
Treasurer Expenses		683.43	
Affiliate Memberships		15.00	
Banking Fees		30.00	
MC - VISA Fees		585.10	
Incorporation Fee		20.00	
Insurance/Bond Fees		600.00	
Refunds		250.00	
Managing and Bulletin Editor Expenses		101.40	
Publication Costs		9,885.17	
AMB		7,467.87	
AMS Newsletter		12,417.30	
Student Research Grants		1,957.00	
1997 Abstracts Mailing		3,017.07	
1997 Council Meeting Expenses		353.43	
1997 Auction Expenses		188.00	
1998 Meeting Expenses - Separate Account			
1999 Meeting Expenses		560.02	
Officer Travel Expenses (to meetings)		4,644.71	
Student Paper Awards		1,500.00	
NET GAIN IN 1998			$22,223.48
ENDING BALANCE			$174,042.52

**The $12,279.50 WCM meeting surplus includes the $7,740.04 surplus from the 1996 AMU meetings in Chicago. In 1997 AMU Council voted to use these surplus funds to support the symposia for the WCM. In 1999 AMS Council recommended that the $7,740.04 be subtracted from the $12,279.50 WCM surplus. Then AMS Council voted to divide the remainder ($4,539.46) with UNITAS. UNITAS was the cosponsor of the WCM in 1998. UNITAS will receive $2,269,73.

66th ANNUAL MEETING
THE AMERICAN MALACOLOGICAL SOCIETY
SAN FRANCISCO, CALIFORNIA
JULY 7 - 12, 2000

The 2000 meeting of the American Malacological Society will be held at San Francisco State University from Friday, July 7 to Wednesday, July 12. The meeting will be held jointly with the 33rd annual meeting of the Western Society of Malacologists.

AMS will sponsor a major symposium entitled "The Place of Malacology in Comparative Biology" to be convened by Michael T. Ghiselin, California Academy Sciences, Golden Gate Park, San Francisco, CA 94118 (mghiselin@calacademy.org; phone (415) 750-7084; FAX 415-750-7090). This symposium will focus on the many historical and contemporary contributions of malacology to other disciplines of biology, including mollusks as model systems.

The meeting will include a banquet at the California Academy of Sciences. Optional field trips, including excursions to Mt. San Bruno with Neil Fahy to look for land snails, Monterey Bay Aquarium or Pt. Reyes National Seashore, Muir Woods National Monument, and Mt. Tamalpais, will be available for participants to attend following the conclusion of formal sessions.

Housing will be available in several categories at San Francisco State University. The accommodations are designed to provide affordable housing to students as well as hotel-style private rooms with daily maid service. All housing options include breakfast, with options for a full meal plan.

For further information please contact:
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Each original manuscript and accompanying illustrations must be submitted with two additional copies for review purposes. Text must be typed on one side of 8-1/2 x 11 inch bond paper, double-spaced, and all pages numbered consecutively with numbers appearing in the upper right hand corner of each page. Leave ample margins on all sides.

Form of the manuscript should follow that outlined in the *Council of Biology Editors Style Manual* (sixth edition, 1994). This can be purchased from the CBE, 11 S. LaSalle Street, Suite 1400, Chicago, IL 60603, U.S.A.

Text, when appropriate, should be arranged in sections as follows:

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3. Text of manuscript starting with a brief introduction followed by methodology, results, and discussion. Separate sections of text with centered subtitles in capital letters.
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All binomens must include the author and date attributed to that taxon the first time the name appears in the manuscript [e. g. *Crassostrea virginica* (Gmelin, 1791)]. This includes nonmolluscan taxa. The full generic name along with specific epithet should be written out the first time that taxon is referred to in each paragragh. The generic name can be abbreviated in the remainder of the paragraph as follows: C. *virginica.*

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Hillis, D. M. 1989. Genetic consequences of partial self fertilization on populations of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). *American Malacological Bulletin* 7(1):7-12.

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Yonge, C. M. and T. E. Thompson. 1976. *Living Marine Molluscs.* William Collins Son and Co., Ltd., London. 288 pp.

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SYMPOSIUM: NEW LOOKS AT OLD MOLLUSCS: RECENT PERSPECTIVES OF MOLLUSCAN EVOLUTION

Organized by

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AMERICAN MALACOLOGICAL SOCIETY
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Is the Aplacophora monophyletic? A cladistic point of view

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Abstract: The systematic position and monophyly (versus paraphyly) of the two aplacophoran taxa, Solenogastres (also called Neomeniomorpha or Ventroplicida) and Caudofoveata (or Chaetodermomorpha) has been a subject of long debate. Also the plesiomorphic versus apomorphic (paedomorphic) condition of several aplacophoran features has been argued for over a century. A cladistic analysis has been undertaken to address these questions or at least to identify the specific lack of knowledge and necessary studies. Outgroup comparison of character states is generally limited, because many relevant organ systems (*e. g.* buccal apparatus, mantle cavity, gonopericardial system, osphradia) do not exist in any possible molluscan outgroup; thus the polarity of these characters cannot be inferred *a priori* to the analysis. Based on current knowledge and available data, the arrangement {Solenogastres [Caudofoveata (Polyplacophora and Conchifera)]} is the most parsimonious one, and the Aplacophora form a basic, paraphyletic assemblage. Accordingly, several aplacophoran features can be reasonably regarded as plesiomorphic for Mollusca. The monophyly of Testaria (Polyplacophora and Conchifera) is very well supported by several characters, contradicting recent ideas about a monophyly of Aculifera (Polyplacophora and Aplacophora). In particular ontogenetic data are badly needed to improve the understanding of aplacophoran relationships and of the ancestral features of the Mollusca as a whole.

Key Words: Mollusca, Aplacophora, phylogeny, inapplicable characters

The origins and interrelationships of the Mollusca and its seven to eight extant primary clades ("classes") has been a matter of debate over a century. Detailed historical reviews have been provided by various authors (*e. g.* Ghiselin, 1988; Haszprunar, 1996a). This contribution focuses on the most recent discussions.

Based on morphological and molecular characters the systematic position of the phylum Mollusca is now fairly well settled among the Spiralia (taxa with spiral-quartet cleavage) or in more recent reviews (*e. g.* Adoutte *et al.*, 2000) among the "Lophotrochozoa" (taxa with ciliary trochi or a lophophore). The long-lasting debate between "turbellarian-like" versus "annelid-like" ancestors has been resolved in favour of a coelomate (*i. e.*, derived from 4d-blastomere, but not eucoelomate, *e. g.*, Salvini-Plawen and Bartolomaeus, 1995) molluscan condition and a non-segmented body plan - at least in the opinion of the present and most recent authors (*e. g.* Haszprunar, 1996a; Haszprunar and Schaefer, 1997b; Haszprunar and Wanninger, 2000; Table 1). Whereas molecular studies remain ambiguous, analyses of phenotypical characters suggested the Kamptozoa (Entoprocta) or the Sipuncula as the direct sister group of the Mollusca (Haszprunar, 1996a; Scheltema, 1993, 1996; but see Jenner and Schram, 1999).

Broad agreement exists on the monophyly of all conchiferan molluscan classes, Monoplacophora (Tryblidia), Bivalvia, Scaphopoda, Gastropoda, and Cephalopoda. However, there is still considerable debate about the status and phylogenetic position of the aculiferan taxa, Caudofoveata (Chaetodermomorpha), Solenogastres (Neomeniomorpha), and Polyplacophora (Neoloricata). Two major points of view have been outlined recently (Fig. 1).

Scheltema (1988, 1993, 1996) and Ivanov (1996) consider the Aculifera a monophyletic taxon which is the sister group of the Conchifera. Within the Aculifera, Chaetodermomorpha and Neomeniomorpha constitute the monophyletic Aplacophora. Scheltema (1988, 1993, 1996) considered the latter taxa as partly progenetic (or paedomorphic) and thus derived. Contrary to that view Salvini-Plawen (1972, 1980, 1981, 1985, 1988, 1991; Salvini-Plawen and Steiner, 1996) regarded Aplacophora and Aculifera as paraphyletic assemblages. The recent cladistic analysis of the latter authors revealed a most parsimonious tree with a polytomy of Solenogastres, Caudofoveata, and Testaria (Polyplacophora and Conchifera). Both authors consider many conditions of the aplacophoran taxa as plesiomorphic for Mollusca rather than derived through paedomorphosis.

Recent progress has been made concerning certain relevant molluscan characters as well as concerning cladistic theory with respect to inapplicable characters (see

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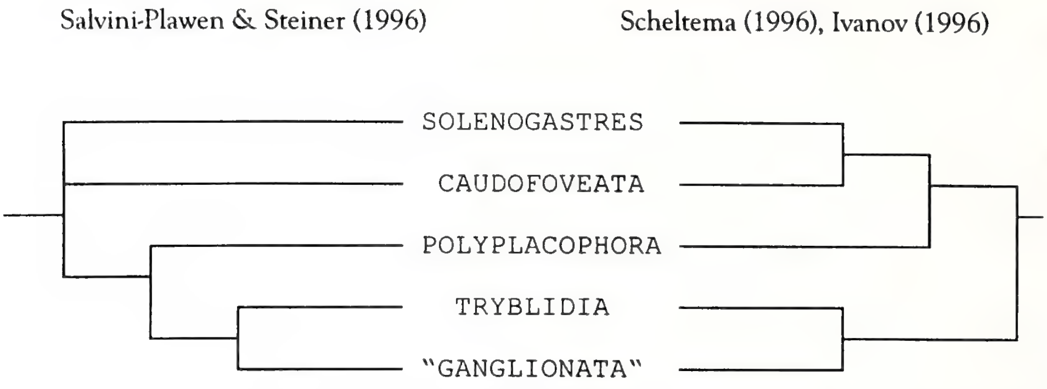
Salvini-Plawen & Steiner (1996)
Scheltema (1996), Ivanov (1996)
SOLENOGASTRES
CAUDOFOVEATA
POLYPLACOPHORA
TRYBLIDIA
"GANGLIONATA"

Fig. 1. Current phylogenetic hypothesis on aplacophoran and aculiferan Mollusca. Left: Preferred tree by Salvini-Plawen and Steiner (1996; their most parsimonious tree shows Solenogastres as the first offshoot). The proposed tree by Waller (1998) is similar, but regards Solenogastres and Caudofoveata (Aplacophora) as monophyletic. Right: Tree based on Scheltema (1996) and Ivanov (1996).

below). Accordingly the present cladistic analysis focuses on (1) the still poor knowledge of the aplacophoran taxa and (2) the problem of correct coding of inapplicable characters in molluscan phylogeny.

METHODOLOGY

Outgroups. All current taxa for consideration as molluscan sister-groups have been coded as outgroups: Kamptozoa (= Entoprocta; cf. Bartolomaeus, 1993; Haszprunar, 1996a), Sipuncula (*cf.* Scheltema, 1993, 1996 in reviving Gerould, 1907), and Annelida (*e. g.* Götting, 1980a, b).

Ingroups. All traditional, extant molluscan "classes" were taken as ingroups, with both Caudofoveata and Solenogastres separately coded. Although monophyly has been discussed in Bivalvia (*e. g.* Gustafson, 1987; Adamkewicz *et al.*, 1997; Steiner and Müller, 1996; Giribet and Carranza, 1999; Steiner, 1999) or Gastropoda (*e. g.* Ponder and Lindberg, 1997), they are regarded here as monophyletic in the strict sense.

Character selection and weighting. Character selection has been identified as the heaviest way of character weighting (Haszprunar, 1998). Selection of characters mostly follows the recent papers by Salvini-Plawen and Steiner (1996) and Waller (1998), although there are some modifications of the coding, which will be discussed. Reasons are given for those omitted characters that have been discussed with respect to the Aplacophora-problem. All selected characters were weighted with equal weight, because several attempts with unequal weighting did not result in a different tree topology. In general the most plesiomorphic state of a class was taken, but if this is ambiguous, the multistate option has been applied.

Proposed aplacophoran synapomorphies. Apart from the parsimony analysis the synapomorphies proposed by authors in favor of a monophyly of the Aplacophora

Table 1. Proposed synapomorphies of the Aplacophora and their distribution within Mollusca and outgroups. The table shows that none of the shared aplacophoran characters can be reasonably considered as apomorphic *a priori* to a cladistic analysis.

Character	APLACOPHORA	TESTARIA (POLYPLACOPHORA & CONCHIFERA)	KAMPTOZOA, SIPUNCULA, ANNELIDA	CONCLUSION FOR MOLLUSCA
Vermiform body	present (1)	only aberrant species (0->1)	present (1)	plesiomorphic
Body wall musculature	present (1)	absent (0)	present (1)	plesiomorphic
Intercrossing d-v muscles	many (0)	few (1)	lacking (?)	equivocal
Specific circumoral field	present (1)	present (1)	present (1)	uninformative
Foot	reduced (1)	present (0)	lacking (?)	equivocal
Spiculoblasts	unicellular (1)	uni- and multicellular (0)	lacking (?)	equivocal
Shell-field	lacking (0)	present (1)	lacking (0)	plesiomorphic
Coelomoducts	one pair (1)	several or one pair (0/1)	many, one or no pair (0/1)	uninformative
Gametes through pericardium	present (1)	absent (0)	no pericardium (?)	equivocal
Mantle cavity	posterior (1)	circumpedal (0)	no mantle cavity (?)	equivocal
Osphradium	extrapallial (1)	intrapallial (0)	no osphradium (?)	equivocal
Cerebral ganglia	present (1)	present or absent (0/1)	present (1)	uninformative
Radula	basically distichous (1)	basically many teeth (0)	no radula (?)	equivocal

were specifically listed and directly evaluated by means of outgroup comparison (Table 1). Doing this the "red-blue-tail" problem becomes crucial (see below).

Red-blue-tail problem. Concerning the coding of certain characters, the so-called "red-blue-tail" problem, *i. e.*, the problem of coding of inapplicable characters (Maddison, 1993; Nelson and Ladiges, 1993; Wilkinson, 1995; Hawkins *et al.*, 1997, Kitching *et al.*, 1998, Lee, 1999) turned out to be of crucial importance, in particular with respect to the outgroups and thus directly associated with (so far nearly exclusively done) *a priori* estimation of the polarity of characters within the Mollusca.

The problem concerns many significant characters in molluscan evolution, namely the type of body cuticle (#2), presence of the periostracal groove (#6), type of mantle cavity (#9), number of ctenidia (#11), number of intercrossing dorso-ventral muscles (#15), details of the pericardioducts (#25, #26) and gamete release (#34), various aspects of the radular apparatus (#38, #39, #40), pedal ganglia (#49), and position of the osphradium (#56). The specific point concerning the Aplacophora-question is outlined in the following with the example of the various aspects of the radular apparatus (#38, #39, #40).

There is large agreement that the two aplacophoran taxa share a similar radular type, regardless whether it is regarded as a strictly distichous or a monoserial dicuspid radula (see Salvini-Plawen, 1988:355-359 for detailed discussion). In contrast, Polyplacophora and the conchiferan taxa share a polystichous rasping tongue for grazing with several to many teeth per row. None of the outgroups possess a radula, thus it would be indeed nonsense to ask which type of radula would have been present, if there would be a radula at all. Ontogenetic patterns of ingroups also do not solve the problem: there is an early stage in the ontogeny of the chiton radula (Sirenko and Minichev, 1975; Salvini-Plawen, 1988: 365) resembling the aplacophoran type. However, one could interpret this similarity either as a recapitulation of the aplacophoran type in the chiton ontogenesis, or assume with equal *a priori* likelihood a paedomorphic event for the aplacophoran taxa from a chiton-like predecessor.

Thus, in this analysis it remains open which type, the dicuspid/distichous or the rasping type, is the plesiomorphic condition for Mollusca. If the rasping type is plesiomorphic, the dicuspid / distichous one would be a synapomorphy of Aplacophora; in the opposite case the rasping tongue would serve as a synapomorphy for Testaria (Polyplacophora and Conchifera). In the given situation it is impossible to infer character polarity *a priori* to the phylogenetic reconstruction. Nevertheless, the character is not useless for the Aplacophora-problem, because the given distribution makes many possible arrangements of the four-taxa problem such as [(Caudofoveata and Polyplacophora) (Solenogastres and Conchifera)] being less parsimonious than others such as {Caudofoveata [Solenogastres (Polyplacophora and Conchifera)]} or {Conchifera [Polyplacophora [Caudofoveata and Solenogastres)]}. Moreover, if either Aplacophora or Testaria turn out to be paraphyletic based on other characters, reasonable inference of character polarity is possible *a posteriori* of tree calculation.

The various authors recommend different ways out of the problem of inapplicable characters. One possibility is to calculate trees with all possible character states in all cases of inapplicable characters. In the present analysis this would result in trillions of calculations (15 characters, three outgroups, *i. e.*, $2^{15 \times 3}$ tree calculations) which were far beyond the computer capacity available. To omit such characters from the analysis would strongly influence the most parsimonious tree topology. Herein, inapplicable states are scored with an "x," which is treated by PAUP as equal to "?" (unknown) (cf. Strong and Lipscomb, 1999).

Software and options. The inference of the most parsimonious tree followed the standard procedure: PAUP 4.0 (Beta-Version; Swofford and Begle, 1999) was applied as the parsimony software, input files were done in the NEXUS format. The ACCTRAN option was selected for tree calculation. Tree calculation was followed by a thorough a posteriori analysis of characters (see Discussion Part).

CHARACTER ANALYSIS

A summary of character coding and the matrix are presented in Table 2. Taxa are abbreviated as follows: Ann - Annelida; Biv - Bivalvia, Cau - Caudofoveata, Cep - Cephalopoda, Gas - Gastropoda, Kam - Kamptozoa (= Entoprocta), Pol - Polyplacophora, Sca - Scaphopoda, Sip - Sipuncula, Sol - Solenogastres, Try - Tryblidia. Taxon/p means "taxon partim."

#1. Cuticle: (0) = absent: Try, Biv, Sca, Gas, Cep; (1) = present: Ann, Sip, Kam, Sol, Cau, Pol..

The condition of the unspecialized dorsal epidermis of the adult is scored.

#2. Type of cuticle: (0) = chitinous: Kam, Sol, Cau, Pol; (1) = collagenous: Ann, Sip; (x) = cuticle absent: Try, Biv, Sca, Gas, Cep.

Coding of this character has been outlined by Haszprunar (1996a).

#3. Aragonitic scales or spicules: (0) = absent: Ann, Sip, Kam, Try, Biv, Sca, Gas, Cep; (1) = present: Sol, Cau, Pol.

All three aculiferan taxa have solitary scale- or spicule-building cells; in addition, the Polyplacophora also have multicellular spiculoblasts.

#4. Shell: (0) = absent: Ann, Sip, Kam, Sol, Cau;

Table 2. Character coding and data matrix.

#1: Cuticle [0 = absent, 1 = present]
#2: Cuticle type [0 = chitinous, 1 = collagenous, x = cuticle absent]
#3: Aculiferan condition [0 = absent, 1 = present]
#4: Shell [0 = absent, 1 = shell plates, 2 = shell by shell gland]
#5: Periostracum [0 = absent, 1 = present]
#6: Periostracal groove [0 = absent, 1 = present, x = no periostracum]
#7: Mantle papillae [0 = absent, 1 = present]
#8: Mantle cavity [0 = absent, 1 = present]
#9: Position of mantle cavity [0 = circumpedal, 1 = posterior, x = no mantle cavity]
#10: Ctenidia [0 = absent, 1 = present]
#11: Number of ctenidia [0 = 1 pair,1 = 2, 2 = 3 to 6, 3 = > 6, x = no ctenidia]
#12: Body wall musculature [0 = ring/diagonal/longitudinal, 1 = otherwise]
#13: Longitudinal body muscles [0 = smooth, 1 = striated, x = no longitudinal muscles]
#14: Intercrossing dorsoventral muscles (IDVM) [0 = absent, 1 = present]
#15: Number of IDVMs [0 = many, 1 = eight, 2 = less than eight, 3= less than three, x = no IDVM] ORDERED
#16: Hydrostatic muscle system [0 = absent, 1 = present]
#17. Specific head retractor: [0 = absent, 1 = present]
#18: Pedal digging by hemolymph pressure [0 = absent, 1 = present]
#19: Pedal gland [0 = absent, 1 = present]
#20: Pedal cirri [0 = absent, 1 = present]
#21: Coelomatic cavity (histological sense) [0 = absent, 1 = present]
#22: Eucoelomate condition [0 = absent, 1 = present]
#23: Heart with pericardium [0 = absent, 1 = present]
#24: Circulatory system [0 = pseudovessels, 1 = endothelial, 2 = sinusial]
#25: Pericardioducts [0 = absent, 1 = present, x = no pericardium]
#26: Formation of Coelomoduct [0 = ingrowth, 1 = outgrowth, x = no coelomoduct]
#27: Number of Coelomoducts [0 = one pair, 1 = two pairs, 2 = more than two pairs, x = no coelomoduct]
#28: Podocytes [0 = absent, 1 = present]
#29: Protonephridia [0 = absent, 1 = present]
#30: Rhogocytes [0 = absent, 1 = present]
#31: Number of gonads [0 = single "right" (pretorsional left), 1 = single right, 2 = one pair, 3 = two pairs, 4 = more than two pairs]
#32: Position of gonad [0 = dorsal to gut; 1 = ventral to gut; ? = not clear]
#33: Urinogenital [0 = absent, 1 = present]
#34: Gamete release through pericardium [0 = absent, 1 = present]
#35: Molluscan Cross [0 = absent, 1 = present]
#36: Jaws [0 = absent, 1 = present]
#37: Radula [0 = absent, 1 = present]
#38: Radular membrane [0 = absent, 1 = present, x = radula lacking]
#39: Rasping tongue [0 = absent, 1 = present, x = radula lacking]
#40: Buccal cartilage [0 = absent, 1 = present, x = no radula]
#41: Oesophageal pouches [0 = absent, 1 = present]
#42: Highly glandular midgut [0 = no, 1 = yes]
#43: Subdivided midgut for sorting and uptake of food [0 = absent, 1 = present]
#44: Bilobed midgut gland [0 = absent, 1 = present, x = no midgut gland]
#45: Crystalline style [0 = absent, 1 = present]
#46: Intestinal loops [0 = absent, 1 = longitudinal, 2 = unidirectional, 3 = bidirectional]
#47: Position of anus [0 = opposite of oral opening, 1= near mouth opening at dorsal side, 2 = near dorsal opening at ventral side]
#48: Tetraneury [0 = absent, 1 = present]
#49: Precerebral ganglia [0 = absent, 1 = present]
#50 Pedal ganglia [0 = absent, 1 = present, x = no pedal nervous system]
#51: Visceral loop and IDVM [0 = between, 1 = outside, 2 = inside, x = no IDVM]
#52: Visceral commissure [0 = suprarectal, 1 = subrectal, x = homology unclear]
#53: Innervation of the shell(-plate) margin. [0 = also visceral, 1 = only cerebropleural, x = no shell-plate]
#54: Cerebral eyes [0 = absent, 1 = present]
#55: Statocysts [0 = absent, 1 = present]
#56: Osphradia [0 = absent, 1 = present]
#57: Position of osphradia [0 = pallial, 1 = extrapallial, x = no osphradium]
#58: Subradular organ [0 = absent, 1 = present]

(1) = shell plates: Pol; (2) = shell by shell gland: Try, Biv, Sca, Gas, Cep.

The homology between the polyplacophoran shell-plates and the conchiferan shell is doubtful (*e. g.* Haas, 1981), therefore both stages are coded.

#5. Periostracum: (0) = absent: Ann, Sip, Kam, Sol, Cau; (1) = present: Pol, Try, Biv, Sca, Gas, Cep.

Periostracum is meant here in a very general way, namely as a purely organic layer covering a shell or shell plate.

#6. Periostracal groove: (0) = absent: Pol; (1) = present: Try, Biv, Sca, Gas, Cep; (x) = no periostracum: Ann, Sip, Kam, Sol, Cau.

Recent fine-structural investigations (Schaefer and Haszprunar, 1997b) revealed significant differences of the organization of the mantle margin between Neopilinidae and other conchiferans. Nevertheless the periostracal groove itself is regarded as homologous throughout the Conchifera.

#7. Mantle papillae: (0) = absent: Ann, Sip, Kam, Try, Biv, Sca, Gas/p, Cep; (1) = present: Sol, Cau, Pol, Gas/p.

Reindl and Haszprunar (1996a,b; Reindl *et al.*, 1995, 1997) investigated the fine-structure and immunocytochemistry of shell pore contents (so-called papillae and caeca) of various molluscan groups and compared them among each other and with the caeca of articulate Brachiopoda. There is a striking similarity between polyplacophoran aesthetes and brachiopod caeca, whereas the bivalve caeca show entirely different structure and mode of formation.

The available data (Hoffmann 1949, Fischer *et al.* 1980, 1988; Scheltema *et al.* 1994) and personal, unpublished fine-structural (TEM) studies on the mantle papillae of Solenogastres and Caudofoveata cannot exclude a possible homology between these papillae and the polypla-

Table 2. Continued

```
MATRIX
                            1                                2                            3                                4                        5
character       12345 67890 1  23  4  5  6  7  8  90 12345 6  7  8  9  0  1  2  3  4  5 67890 1234  5  678  9  0 12345 678

Annelida        11000 x00x0 x  01  0  x  0  0  0  00 1100x 0  2  1{0,1}0  4  ?  0  x  0 10xxx 000x  0  000  0  0 xxx10 0x0
Sipuncula       11000 x00x0 x  01  0  x  0  x  0  00 1100x 0  0  1  0  0  2  ?  0  x  1 00xxx 000x  0  110  0  0 xxx10 0x0
Kamptozoa       10000 x00x0 x  1x  0  x  0  x  0  01 0001x x  x  0  1  0  2  0  1  x  0 00xxx 010x  0  01?  0  x xxx10 0x0
Solenogastres   10100 x1110 x  00  1  0  0  0  0  11 10111 ?  0  1  ?  1  2  0  0{0,1}1 01000 010x  0  001{0,1}0 00x00 110
Caudofoveata    10100 x1111 0  00{0,1}0  0  0  0  00 10111 ?  0  1  ?  1  2  0  0  1  ? 01100 0110{0,1}001  1  0 00x00 110
Polyplacophora  10111 01101 3  1x  1  1  0  0  0  10 10111 1  0  1  1  1  2  0  1  0  1 01111 1111  0  301  0  0 10000 101
Tryblidia       0x021 10101 2  1x  1  1  0  0  0  00 10110 ?  2  1  ?  1{2,3}1  0  0  ? 11111 1111  1  201  0  0 11001 0x1
Bivalvia        0x021 10101 0  1x  1  2  0  0  1  00 10111 1  0  1  1  1  2  0  0  0  1 00xxx 1111  1  301  0  1 11001 100
Scaphopoda      0x021 10100 x  1x  1  3{0,1}0{0,1}00 10011 ?  0  1  1  1  1  0  0  0  1 11111 1111  0  321  0  1 11001 0x1
Gastropoda      0x021 10111 0  1x  1  3  1  1  0  10 10111 1  0  1  1  1  0{0,1}0  0  1 11111 1111{0,1}321  0  0 21111 101
Cephalopoda     0x021 10111{0,1}1x ?  3  1  1  0  00 10121 1{0,1}1  0  1{1~3}0  1  0  0 11111 0111  0  321  0  1 21111 101
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cophoran macroaesthetes, although there are no distinct similarities as in the case of the Brachiopoda. However, if homology is assumed, then the fissurellid (but not bivalvian) caeca need to be included, accordingly the Gastropoda are scored by {0,1}.

#8. Mantle cavity: (0) = absent: Ann, Sip, Kam; (1) = present: Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep.

The reduction and loss of the mantle cavity in various gastropod and certain bivalve taxa is considered as a secondary matter of multiple convergence, therefore both are coded (1).

#9. Position of mantle cavity: (0) = circumpedal: Pol, Try, Biv, Sca; (1) = posterior: Sol, Cau, Gas, Cep; (x) = no mantle cavity: Ann, Sip, Kam.

Hoffmann (1949) and Salvini-Plawen (*e. g.* 1981, 1991) considered the mantle cavity of the Solenogastres as reduced and narrowed, yet of the circumpedal type found in Polyplacophora, Tryblidia, Bivalvia, and Scaphopoda. In all the latter groups the visceral nerve cord surrounds the dorsoventral (shell-) muscle bundles and directly innervates the mantle epithelium and the various organs. In contrast, the visceral cords of Solenogastres run between the two pairs of dorsoventral muscles (see Haszprunar, 1989), and innervation of the peripedal groove has never been shown. Thus, there is no reason to homologize the peripedal groove between the foot-sole and the cuticularized mantle with a mantle cavity. The mantle cavity of Solenogastres is therefore coded as "posterior" (1).

The neural condition of the Caudofoveata is less clear because of the reduction of the dorsoventral muscles. However, there is no doubt that the caudofoveate mantle cavity is purely posterior.

The anlage of the gastropod mantle cavity is situated posteriorly, therefore gastropods are scored by (1).

Because the character is inapplicable in all outgroups the coding of the circumpedal and posterior stage with (0) or (1) does not imply any decision concerning polarity.

#10. Ctenidia: (0) = absent: Ann, Sip, Kam, Sol, Sca; (1) = present: Cau, Pol, Try, Biv, Gas, Cep.

In recent times Morton (1988), Lindberg (1989), and also Ponder and Lindberg (1997: 112-113) expressed doubts about the homology of the ctenidia between the molluscan classes. Whereas there is high probability that the respiratory surfaces of the ctenidium evolved several times in molluscan evolution, the common ancestry of this originally ventilatory organ (*i. e.,* causing water current; see Haszprunar, 1992) is well supported by shared position, structure, and innervation (see Haszprunar, 1987a: fig. 5).

All authors agree that lack of ctenidia is a secondary phenomenon in the Gastropoda, therefore they are coded by (1).

#11. Number of ctenidial pairs: (0) = 1 pair: Cau, Biv, Gas, Cep/p; (1) = 2 pairs: Cep/p; (2) = 3 to 6 pairs: Try; (3) = more than 6 pairs: Pol; (x) = no ctenidia: Ann, Sip, Kam, Sca.

Recent, unpublished observation on *Micropilina minuta* revealed the presence of four pairs of ctenidia, so that the Tryblidia exhibit a continuous range from 3 to 6 ctenidial pairs.

As outlined by Yonge (1939) the condition of the mantle cavity and ctenidial arrangement differ significantly between the Lepidopleurida and the remaining Polyplacophora (Chitonida). Whereas in the former group the ctenidial number increases with size towards the anal opening, in the Chitonida the ctenidia multiply forwards when becoming larger. Accordingly the multiplication of ctenidia is an independent matter in Lepidopleurida and Chitonida and is not used as a synapomorphy of the Polyplacophora. Because of these circumstances all character states are coded as unordered.

#12. Body wall musculature: (0) = circular/diagonal/longitudinal: Ann, Sip, Sol, Cau; 1 = otherwise: Kam, Pol, Try, Biv, Sca, Gas, Cep.

This character replaces the more obscure "worm-like shape" by an observable character, *i. e.*, the presence of a distinct body wall musculature, which is composed of outer circular, intermediate diagonal, and inner longitudinal muscle fibers. This condition is typical for Sipuncula and Polychaeta; among the Mollusca it exists solely in the aplacophoran taxa. Similar conditions in various groups of opisthobranch or pulmonate slugs are without doubt secondary conditions, because all earlier (5 to 10) gastropod clades lack this condition (Haszprunar, 1988; Ponder and Lindberg, 1997).

#13. Structure of the longitudinal muscles of body wall: (0) = smooth: Sol, Cau; (1) = striated: Ann, Sip; (x) = no longitudinal muscles: Kam, Pol, Try, Biv, Sca, Gas, Cep.

Annelids and sipunculans are known to have obliquely striated longitudinal muscles, whereas those in the aplacophoran taxa are smooth. For all other taxa the character is inapplicable.

Contrary to Salvini-Plawen (1981, 1991) I regard the homology of the longitudinal enrolling muscles in Polyplacophora and Solenogastres as doubtful. The enrolling muscles of Solenogastres and Caudofoveata are a specialized, latero-ventral part of the longitudinal layer of the body wall musculature. Recent studies on the myogenesis of the chiton *Mopalia muscosa* revealed that the enrolling muscle is in principle a ring-system independent of the original body wall muscle grid, and that the enrolling function is provided by the transverse stiffness of the shell-plates (Haszprunar and Wanninger, 2000). Purely pedal position and innervation also exclude homology of both laterally situated and innervated enrolling muscles with the suctorial muscle of extant Monoplacophora.

#14. Intercrossing of the inner dorsoventral musculature (IDVM): (0) = absent: Ann, Sip, Kam; (1) = present: Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep.

In contrast to other "worms" the Mollusca are characterized by a ventral intercrossing of the inner muscle bundles of their dorsoventral (shell-) musculature. Because of the lack of a pedal sole this character is missing in most Caudofoveata, but the genus *Scutopus* shows the basic condition (Salvini-Plawen, 1972: fig. 16), therefore coding is {0,1}. Contrary to the statement by Voltzow (1988), Patellogastropoda also show intercrossing dorsoventral muscles (pers. obs.). The conditions in the Cephalopoda are unclear (?) because of the major reconstruction of the foot.

#15. Number of dorsoventral muscle pairs: (0) = many: Sol, Cau; (1) = eight: Pol, Try; (2) = less than eight: Biv; (3) = less than three: Sca, Gas, Cep; (x) = no IDVM: Ann, Sip, Kam.

There is a general tendency to reduce the number of dorso-ventral muscles in the Mollusca (Haszprunar and Wanninger, 2000), particularly exemplified in the Bivalvia, in which extant Protobranchia and Pteriomorpha have seven to three pairs, and Heterodonta usually three. Scaphopoda show one or two pairs (Steiner, 1992). The anterior pair of the cephalopod "depressores infundibuli" is the head retractor (see below), so that Cephalopoda have a single pair. Recent ontogenetic data on the myogenesis of Gastropoda confirmed the presence of a single pair of shell muscles even in cases of secondary splittings such as in *Patella* (Wanninger *et al.*, 1999).

Because this is a continuous series of reductions, it makes sense to code this multistate character as "ordered." Indeed, this option is crucial for the resolution of the conchiferan taxa (see discussion).

#16. Hydrostatic muscular system: (0) = absent: Ann, Sip, Kam, Sol, Cau, Pol, Try, Biv, Sac/p; (1) = present: Sca/p, Gas, Cep.

As outlined by Haszprunar (1988: 405) cephalopods and gastropods share a "hydrostatic muscular system" meaning that extension of body parts or tentacles is caused by muscle contraction analogous to the vertebrate tongue rather than by hemolymphatic pressure. According to Shimek and Steiner (1997) the same is true for the foot of dentaliidan scaphopods explaining the ability of rapid extension and burrowing of the latter organ.

#17. Specific head retractor: (0) = absent: Ann, Sol, Cau, Pol, Try, Biv, Sca; (1) = present: Gas, Cep; (x) = no head: Sip, Kam.

Gastropoda and Cephalopoda share a free head, which is separately retractable by a specific head retractor. Limpets in particular show often a distinct insertion scar of this head retractor, in the Cephalopoda these are the anterior pair of the "depressores infundibuli." Contrary to the statement that also Scaphopoda have a free head (*e. g.* Waller, 1998) the latter have a free buccal cone alone, whereas the remaining head (cerebral and buccal mass) is fixed (Shimek and Steiner, 1997).

#18. Pedal digging by hemolymph pressure: (0) = absent: Ann, Sip, Kam, Sol, Cau, Pol, Try, Sca/p, Gas, Cep; (1) = present: Biv, Sca/p.

Bivalves and gadilidan Scaphopoda (Shimek and Steiner, 1997) use their foot for digging by means of hemolymph pressure in a soft sediment. Caudofoveata show a similar feature but use the cerebrally innervated head-region for digging as secondarily achieved by *e. g.* the naticid caenogastropods (head and foot) or bullomorph opisthobranchs. To the contrary many bivalve taxa secondarily settle on hard substrates by means of a byssus.

#19. Pedal gland: (0) = absent: Ann, Sip, Kam, Cau, Biv, Sca, Cep; (1) = present: Sol, Pol, Gas.

A true pedal gland is herein defined as a subepithelial gland at or near the anterior margin of the foot sole. Accordingly the "pedal glands" of Tryblidia and the "funnel gland" of coleoid cephalopods (*Nautilus* lacks a funnel gland) do not fit this definition, since both consist of purely epithelial mucous cells. Polyplacophora are scored by (1), since a true pedal gland occurs in early juveniles.

The so-called "lip gland" of the sipunculid pelagosphaera larva is a cerebrally innervated structure (Rice, 1993) and thus is not homologous (Scheltema, 1993) to the pedally innervated molluscan gland (Gerould, 1907).

#20. Pedal cirri: (0) = absent: Ann, Sip, Cau, Pol, Try, Biv, Sca, Gas, Cep; (1) = present: Kam, Sol.

Haszprunar (1986; see also Scheltema *et al.* 1994) described the ultrastructure of pedal cirri in the "pedal pit" of certain Solenogastres. Very similar structures occur at the anterior margin of the gliding sole of kamptozoan larvae (Nielsen, 1971; Haszprunar *et al.*, 1995), although fine-structural studies are still lacking.

#21. Coelomatic cavities: (0) = absent: Kam; (1) = present: Ann, Sip, Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep.

There is no doubt that molluscs are coelomate in the histological and embryological sense, *i. e.*, there are mesodermal epithelial cavities, namely the gonopericardial system, out of the 4d-blastomere.

#22. Eucoelomate condition: (0) = absent: Kam, Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep; (1) = present: Ann, Sip.

Bartolomaeus (1993, 1994, Salvini-Plawen and Bartolomaeus, 1995, Haszprunar, 1996a, b) defined the eucoelomatic condition (#22) by the checkable feature that the inner wall of the coelomic cavity forms the epitheliomuscular layer of the gut. This condition is not present in any mollusc, but is (among many other phyla) found in Sipuncula and Annelida.

#23. Heart in pericardium: (0) = absent: Ann, Sip, Kam, Sca; (1) = present: Sol, Cau, Pol, Try, Biv, Gas, Cep.

The specific structures of the molluscan heart are unique and constitute a synapomorphy for the phylum. One known tryblidian genus (*Micropilina*), the Scaphopoda, and certain opisthobranchs (*e. g. Alderia modesta, Rhodope* spp.) have lost the heart secondarily. Scaphopoda are unique in retaining at least the pericardial cavity and its function concerning ultrafiltration (Reynolds, 1990).

#24. Circulatory system: (0) = pseudovessels: Ann, Sip; (1) = mainly sinusial: Kam, Sol, Cau, Pol, Try, Biv, Sca, Gas; (2) = mainly endothelial: Cep.

As outlined by Bartolomaeus (1993) and Haszprunar (1996a), Kamptozoa and Mollusca share a circulatory system of sinuses. Pseudovessels, *i. e.*, a lining by outwards orientated epithelia, are found in most eucoelomates (here Sipuncula and Annelida), whereas true, endothelial vessels occur in certain stylommatophorans (capillaries; *cf.* Luchtel *et al.* 1997) and cephalopods (all except capillaries, *cf.* Budelmann *et al.*, 1997).

#25. Pericardioduct: (0) = absent: Try; (1) = present: Sol, Cau, Pol, Biv, Sca, Gas, Cep; (x) = no pericardium: Ann, Sip, Kam.

With the notable exception of Tryblidia (Haszprunar and Schaefer, 1997a; Schaefer and Haszprunar, 1997a), all molluscs that possess a pericardial cavity also have a pericardioduct releasing the modified primary ultrafiltration product into the mantle cavity. Because a true pericardium is lacking in all outgroups, they are scored by (x).

#26. Formation of coelomoducts: (0) = ingrowth: Ann, Sip; (1) = outgrowth: Pol, Biv, Gas, Cep; (x) = no coelomoduct: Kam; (?) = unknown: Sol, Cau, Try, Sca.

As outlined in detail by Bartolomaeus (1993, 1994) and Salvini-Plawen and Bartolomaeus (1995) the nephridial ducts in molluscs (Polyplacophora, Bivalvia, Gastropoda, and Cephalopoda; no data on the remaining classes) are formed by outgrowth of the coelomic cavity, whereas the (gono-)nephridial ducts in eucoelomates are formed by epidermal ingrowth.

According to Baba (1938), in the solenogastre *Epimenia* "there arise on the neck of proctodaeum a pair of short diverticula which may develop into gonoducts [*i. e.*, pericardial = urinogenital ducts]." However, the proctodaeum itself is an outgrowth of the endodermal mass and not an epidermal infolding. I still code the solenogastre condition as unknown.

#27. Number of coelomoducts: (0) = one: Sip, Sol, Cau, Biv, Sca, Cep/p; (1) = two: Cep/p; (2) = more than two: Ann, Try; (x) = no coelomoduct: Kam.

Among the Mollusca only *Nautilus* shows two pairs of coelomoducts. If (as is done here) the excretory organs are also considered as coelomic cavities (see above), the extant Tryblidia are characterized by several (three to seven) coelomoducts.

#28. Podocytes: (0) = absent: Ann/p, Kam; (1) = present: Ann/p, Sip, Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep.

Podocytes have been described in all molluscan classes. Adults of certain polychaete taxa have solenocytes instead of podocytes (*e. g.* the recent review by Bartolomaeus, 1999).

#29. Protonephridia: (0) = absent: Sip; Cep; (1) =

present: Ann, Kam, Pol, Biv, Sca, Gas; (?) = unknown: Sol, Cau, Try.

Based on the discovery of protonephridial cyrtocytes in the larva of a chiton, Bartolomaeus (1989) postulated larval protonephridia as a character of the molluscan ground pattern. Recent personal investigations on larvae of *Chiton olivaceus* confirmed the presence of prominent protonephridia also for that species. Moreover, the recent discovery of protonephridia in larvae of the primitive limpet *Patella caerulea* (Haszprunar and Ruthensteiner, 2000) and in the larva of the scaphopod *Antalis vulgatum* (Haszprunar *et al.*, 2000), close former significant gaps of occurrence. However, fine-structural data on aplacophoran larvae are badly needed to determine whether protonephridia are also present in Solenogastres and Caudofoveata.

#30. Rhogocytes (pore cells): (0) = absent: Ann, Sip, Kam; (1) = present: Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep.

Haszprunar (1996a,b) has outlined in detail the significance of the diagnostic molluscan rhogocyte (often called pore-cell) for general nephridial evolution. In addition, the presence of rhogocytes has been confirmed for both aplacophoran taxa (pers. obs.) and scaphopods (G. Steiner, pers. comm.).

#31. Number of Gonads: (0) = single "right" (pretorsional left): Gas; (1) = single right: Sca, Cep/p; (2) = one pair: Sip, Kam, Sol, Cau, Pol, Try/p, Biv, Cep/p; (3) = two pairs: Try/p, Cep/p; (4) = more than two pairs: Ann.

The majority of molluscan classes show a single pair of gonads, but there is gonadal asymmetry in Cephalopoda in part (left side), Scaphopoda in general (right side), and Gastropoda in general (posttorsional right = pretorsional left side) show gonadal asymmetry. *Nautilus* and Neopilinidae (*Micropilina arntzi* has a single pair of gonads: Haszprunar and Schaefer, 1997b) have multiple gonads.

#32. Position of Gonad: (0) = dorsal of gut: Kam, Sol, Cau, Pol, Biv, Sca, Gas/p, Cep; (1) = ventral of gut: Try, Gas/p; (?) = equivocal: Ann, Sip.

Both aplacophoran taxa have dorsal gonads. According to Ponder and Lindberg (1997: 128) "the gonad lies dorsally in polyplacophorans, scaphopods, bivalves, cephalopods, and in all gastropods except patellogastropods, in which it is ventral as in monoplacophorans." Sipuncula and Annelida are equivocal in this character, Kamptozoa clearly have a dorsal gonad.

#33. True gonoducts: (0) = absent: Ann, Sip, Sol, Cau, Try, Biv, Sca, Gas; (1) = present: Kam, Pol, Cep.

Among molluscs true gonoducts are restricted to Polyplacophora and Cephalopoda, although this condition also occurs secondarily in the Bivalvia (*e. g.* Mackie, 1984) and Gastropoda (*e. g.* Haszprunar, 1988; Ponder and Lindberg, 1997). In Solenogastres only the genus *Phyllomenia* possesses true gonoducts (see below).

#34. Release of gametes through the pericardium: (0) = absent: Sol/p, Pol, Try, Biv, Sca, Gas, Cep; (1) = present: Sol/p, Cau; (x) = no pericardium: Ann, Sip, Kam.

Both Salvini-Plawen (1972, 1981, 1985, but see 1991:17) and Scheltema (1996) agree in regarding the passage of gametes through the pericardium as derived. However, whereas Scheltema regards this character as a synapomorphy of Aplacophora, Salvini-Plawen argued in favour of a convergent evolution of this character because of (secondary) elongation and lateral enrolling of the body. The latter view is based on the exceptional condition of the solenogastre genus *Phyllomenia,* in which true gonoducts do exist as well as true pericardioducts (Salvini-Plawen, 1970).

As outlined in the general character analysis, the lack of a heart in all potential outgroups hinders the direct application of the outgroup-criterion for evaluating the polarity of this character. Concerning ingroup comparison and patterning there are three *a priori* possibilities: (1) The conditions in *Phyllomenia* (and in part *Dorymenia*) represent a secondary atavistic reversal to the ancestral molluscan features. This would be the only explanation in regarding "pericardial release of gametes" as a synapomorphy for Aplacophora. However, because of the highly complex genital system of *Phyllomenia* (Salvini-Plawen, 1970), this assumption is very unlikely. (2) *Phyllomenia* conditions represent a retained primitive feature, implying convergent evolution of heart passage at least once in the remaining Solenogastre (if *Phyllomenia* is the sister taxon of them) and in the Caudofoveata. (3) The general aplacophoran condition is plesiomorphic for Mollusca and *Phyllomenia* represents a parallel, derived condition (see Salvini-Plawen, 1991: 17 for similar ideas). If so, the condition "evolution of true gonoducts" is paralleled in the Polyplacophora as a whole and within several lineages of Bivalvia and Gastropoda (see above). I regard this assumption as the most probable view.

For the cladistic analysis Solenogastres were coded {0,1}, since both conditions occur within the taxon and (contrary to Bivalvia and Gastropoda) the plesiomorphic one is not clear.

#35. Cleavage with "molluscan cross": (0) = absent: Ann, Kam, Cep; (1) = present: Sip, Sol, Pol, Biv, Sca, Gas; (?) = unknown: Cau, Try.

For discussion see Haszprunar (1996a).

#36. Jaws: (0) = absent: Sip, Kam, Sol, Cau, Pol, Biv; (1) = present: Ann, Try, Sca, Gas, Cep.

There is agreement that the presence of jaws is a conchiferan character being secondarily lost in Bivalvia and several gastropod taxa.

#37. Radula: (0) = absent: Ann, Sip, Kam, Biv; (1) = present: Sol, Cau, Pol, Try, Sca, Gas, Cep.

There are no convincing arguments in regarding any buccal structure in any annelidan taxon as a homolog of the molluscan radula.

#38. Radular membrane: (0) = absent: Sol; (1) = present: Cau, Pol, Try, Sca, Gas, Cep; (x) = radula lacking: Ann, Sip, Kam, Biv.

A true radular membrane, i.e., a distinct layer below the radular teeth proper (see Scheltema *et al.*, 1994: fig. 19; Eernisse and Reynolds, 1994: fig.11A), is present in all molluscan classes with a radula except the Solenogastres, where TEM-studies (Haszprunar in Salvini-Plawen, 1988; Wolter, 1992) suggest a kind of "pre-ribbon" (Wolter, 1992). It should be mentioned that Scheltema (pers. comm.) still insists in the presence of a true radula membrane at least in certain solenogastre species, unfortunately there is no TEM-evidence for this point of view.

#39. Radular type: (0) = basically distichous/bifid: Sol, Cau; (1) = basically rasping: Pol, Try, Sca, Gas, Cep; (x) = radula lacking: Ann, Sip, Kam, Biv.

For discussion see under general methodology.

#40. Buccal cartilages: (0) = absent: Sol, Cau; (1) = present: Pol, Try, Sca, Gas, Cep; (x) = radula lacking: Ann, Sip, Kam, Biv.

There is a long-lasting and continuing equivocal use of the term "radular bolster" which may be formed either by more or less vacuolized muscle cells or by true cartilages. The latter type is restricted to Polyplacophora and Conchifera, whereas the aplacophoran taxa show the muscular type. Heterobranch Gastropoda secondarily show the purely muscular type again (Haszprunar, 1988; Ponder and Lindberg, 1997).

#41. Oesophageal pouches: (0) = absent: Ann, Sip, Kam, Sol, Cau, Cep; (1) = present: Pol, Try, Biv, Sca, Gas.

As outlined in detail by Salvini-Plawen (1988) the presence of broad and glandular oesophageal pouches with a ciliated, dorsal food channel is typical for Polyplacophora and most conchiferan classes at least in their primitive representatives. The only exception is the Cephalopoda.

#42. Highly glandular midgut: (0) = no: Ann, Sip; (1) = yes: Kam, Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep.

Whereas the midgut of Sipuncula and Annelida is a more or less simple tube, those of Kamptozoa and Mollusca have large glandular areas.

#43. Subdivided midgut for sorting and uptake of food: (0) = absent: Ann, Sip, Kam, Sol; (1) = present: Cau, Pol, Try, Biv, Sca, Gas, Cep.

All Mollusca except the Solenogastres show distinct functional subdivisions of the midgut into a sorting area (stomach), digestion area (midgut sac or gland), and transport tube (intestine). Since none of the outgroups shows this subdivision, it is likely a (syn-?) apomorphic condition of Caudofoveata and all remaining Mollusca.

Salvini-Plawen's (1981, 1988) thorough reviews on the evolution of the molluscan alimentary tract emphasized the differences between the midgut in Caudofoveata and in Testaria. However, having in mind the variability of this region between and within the various conchiferan classes, this seems not a valid argument versus an *a priori* assumption of homology testable by parsimony. To avoid multiple, directly correlated characters, this complex has been taken as a single character, although multiple coding (*e. g.* for intestine, midgut gland, stomach) would be theoretically possible.

#44. Bilobed midgut gland: (0) = absent: Cau; (1) = present: Pol, Try, Biv, Sca, Gas, Cep; (x) = no midgut gland: Ann, Sip, Kam, Sol.

Whereas the midgut sac of the Caudofoveata is a solitary structure, the midgut gland of all other groups is at least bilobed, although secondary asymmetry or multiplication occurs repeatedly among many conchiferan taxa.

#45. Crystalline style: (0) = absent: Ann, Sip, Kam, Sol, Cau/p, Pol, Sca, Gas/p, Cep; (1) = present: Cau/p, Try, Biv, Gas/p.

Chaetodermatid caudofoveates have a gastric shield and a protostyle, whereas the remaining Caudofoveata lack these features. Tryblidia, Bivalvia and many gastropod taxa have so-called crystalline styles or protostyles in their stomach (Salvini-Plawen, 1981, 1988).

#46. Intestinal loops: (0) = absent: Ann, Kam, Sol, Cau; (1) = along longitudinal axis: Sip; (2) unidirectional: Try; (3) true bidirectional looping: Pol, Biv, Sca, Gas, Cep.

Whereas the gut is straight or simply U-shaped in Annelida, Kamptozoa, Solenogastres, and Caudofoveata, intestinal looping is present in the remaining taxa of this study. However, looping is caused by coiling around a longitudinal muscle in Sipuncula, which is not the case in any molluscan taxon. Moreover, the Tryblidia uniquely show unidirectional looping, whereas Polyplacophora, Bivalvia, Scaphopoda, Gastropoda, and Cephalopoda exhibit bidirectional loops.

#47. Position of anus: (0) = opposite of oral opening: Ann, Sol, Cau, Pol, Try, Biv; (1) near mouth opening at dorsal side: Sip, Kam; (2) = near dorsal opening at ventral side: Sca, Gas, Cep.

Ponder and Lindberg (1997) and Waller (1998) have pointed out that Scaphopoda, Gastropoda and Cephalopoda share a so-called "ano-pedal flexure", whereas the remaining molluscan classes the anterio-posterior axis is predominate.

#48. Tetraneury: (0) = absent: Ann, Sip; (1) = present: Sol, Cau, Pol, Try, Biv, Sca, Gas, Cep; (?) = unknown: Kam.

Tetraneury is a typical molluscan character, whereas Sipuncula and Annelida show a single pair of longitudinal main cords. The conditions in the adult Kamptozoa are not relevant, and those of the larva are largely unknown, therefore Kamptozoa are coded by (?).

Scheltema (1993, 1996, Scheltema *et al.*, 1994) considered "ganglionated tetraneury" as a synapomorphy of Aplacophora. However, according to personal observations on several genera of Caudofoveata and Solenogastres the so-called "ganglia" are in many species (but not all; Salvini-Plawen, pers. comm.) just thickenings in the lateral or pedal cords and do not fit the usual definition of ganglia as being interconnected by axons only.

#49. Precerebral Ganglia: (0) = absent: Ann, Sip, Kam, Sol/p, Pol, Try, Biv, Sca, Gas, Cep; (1) = present: Sol/p, Cau.

Precerebral ganglia are generally present in Caudofoveata, whereas they occur only in certain Solenogastres; the latter are therefore coded as {0,1}. Convergent precerebral ganglia are known from a number of interstitial opisthobranchs, but this is clearly a secondary condition in gastropods.

#50. Pedal ganglia: (0) = absent: Ann, Sip, Sol, Cau, Pol, Try, Gas; (1) = present: Biv, Sca, Cep; (x) = no pedal nervous system: Kam.

Among molluscs, true pedal ganglia (versus elongated, pedal cords) are restricted to Bivalvia, Scaphopoda, and Cephalopoda, and they occur as a secondary condition in many gastropod groups.

#51. Position of visceral loop: (0) = between DVM: Sol, Cau; (1) = outwards DVM: Pol, Try, Biv, Sca; (2) = inwards DVM: Gas, Cep; (x) = no DVM: Ann, Sip, Kam.

As outlined earlier (Haszprunar, 1985) the position of the visceral loop (= lateral cord) with respect to the position of the dorsoventral muscles differs significantly between the molluscan classes. In Caudofoveata and Solenogastres the visceral loop runs between the dorsoventral muscle fibers, whereas the visceral loop runs around the shell muscles in the Polyplacophora, Tryblidia, Bivalvia, and Scaphopoda. Gastropoda and Cephalopoda are characterized by a visceral loop lying between the shell muscles enabling these groups to concentrate their nervous system to a great extent. Outgroups lack the specific dorsoventral muscles and are therefore coded as inapplicable (x).

#52. Position of visceral commissure: (0) = suprarectal: Sol, Cau, Pol; (1) = subrectal: Try, Biv, Sca, Gas, Cep; (x) = homology unclear: Ann, Sip, Kam.

The homology of the lateral (visceral) and pedal cord to those of the outgroups is very questionable. Reisinger (1972) regarded the main cords of Sipuncula and Annelida as homologs to the molluscan pedal cord. Considering the suprarectal commissure in aculiferan molluscs and the eucoelomates, it is more likely that the visceral cord is the common one. Because of these uncertainties the outgroups are here coded by (x).

#53. Innervation of the shell(-plate) margin: (0) = cerebropleural and visceral: Pol, Try, Biv, Sca; (1) = only cerebropleural: Gas, Cep; (x) = no shell-plate: Ann, Sip, Kam, Sol, Cau.

This is a new character that has not been considered previously for molluscan relationships. The margin (or growth-zone) of the shell(-plates) of Polyplacophora (Eernisse and Reynolds, 1994), Tryblidia (Lemche and Wingstrand, 1959), Bivalvia (Haas, 1935), and Scaphopoda (Shimek and Steiner, 1997) are innervated by the lateral cords and by the cerebropleural region. In contrast, the shell-margin of Gastropoda (*e. g.* Fretter and Graham, 1962) and Cephalopoda (Young, 1965) are innervated solely by the pleural region or ganglia, but not by the visceral loop. Since the cerebropleural region of gastropods is not involved in the torsion process, the orientation of the adult gastropod shell (teleoconch) is identical to those of the pretorsional ancestor or to the sister group Cephalopoda.

#54. Cerebral (pretrochal) eyes: (0) = absent: Sol, Cau, Pol, Try, Biv, Sca; (1) = present: Ann, Sip, Kam, Gas, Cep.

The homology of metazoan cephalic eyes is still a matter of considerable debate, although there is increasing agreement in favour of multiple convergences despite a common epigenetic "master"-basis (*e. g.* Salvini-Plawen and Mayr, 1977; Salvini-Plawen, 1982; Zuker, 1994; Nilsson, 1996; Gehring and Ikeo, 1999; Meyer-Rochow, 2000). In the Mollusca only Gastropoda and Cephalopoda show cerebrally innervated (*i. e.*, pretrochal) eyes, whereas superficially similar, but pleurally-laterally innervated (*i. e.*, posttrochal) photoreceptive organs in polyplacophoran and bivalve larvae or juveniles do not fulfill the criterion "cerebral".

All outgroups possess cerebral (pretrochal) eyes in the given definition either as adults or at least in the larval stage, although homology is doubtful because of significant differences in their fine-structure (*e. g.* Woollacott and Eakin, 1973; Verger-Bocquet, 1992; Bartolomaeus, 1992; Blumer, 1997).

#55. Paired statocysts: (0) = absent: Ann, Sip, Kam, Sol, Cau, Pol; (1) = present: Try, Biv, Sca, Gas, Cep.

Among molluscs paired statocysts are restricted to the conchiferan classes, although analogous, unpaired gravity receptors have been described in some Solenogastres (Haszprunar, 1986, Scheltema *et al.*, 1994).

#56. Osphradia: (0) = absent: Ann, Sip, Kam, Try, Sca; (1) = present: Sol, Cau, Pol, Biv, Gas, Cep.

Because of the correlation in position and innervation Haszprunar (1987a, b) pointed out the homology of chemoreceptive sensory organs known as "dorsoterminal sense organ", "Geruchsorgan," "Lacaze's Organ," or "osphradium."

#57. Position of osphradia: (0) = pallial: Pol, Biv, Gas, Cep; (1) = extrapallial: Sol, Cau; (x) = no osphradium: Ann, Sip, Kam, Try, Sca.

Both aplacophoran taxa are characterized by extrapallial osphradia (dorsoterminal sense organs), whereas the osphradium of the remaining classes, if it is present, is pallial.

#58. Subradular Sense Organ: (0) = absent: Ann, Sip, Kam, Sol, Cau, Biv; (1) = present: Pol, Try, Sca, Gas, Cep.

Despite the repeated claim of Heath (1904, 1911) that a subradular organ is present in aplacophorans, it is lacking there (*e. g.* Salvini-Plawen, 1978, 1985; Scheltema *et al.*, 1994). The presence of a subradular organ is restricted to Polyplacophora, Tryblidia, Scaphopoda, Cephalopoda (*Nautilus*), and Gastropoda, where it is lost independently in several subclades.

CHARACTERS BEING EXCLUDED FROM THIS ANALYSIS

Cephalic appendages: Although a general cerebral innervation is present, the probability of homology of cephalic appendages between the molluscan taxa and the selected outgroups is extremely low, because their anlagen are placed anteriorly (gastropod tentacles) versus posteriorly (bivalve oral lappets, scaphopod captaculae) of the prototroch. Therefore the present analysis does not consider this character.

Oral lappets: Waller (1998) proposed the lack of oral lappets as a synapomorphy of Scaphopoda, Gastropoda, and Cephalopoda. However, many gastropods have prominent oral lappets, and cephalopods are equipped with oral tentacles. Therefore this character is not scored herein.

Epipodial projections: Homologization of the scaphopod pedal flaps with the originally posterior epipodial tentacles and the cephalopod funnel (Waller, 1998) is extremely doubtful. Whereas the pedal flaps and the funnel are locomotory organs, gastropod epipodial tentacles are sensory structures. In Patellogastropoda the epipodial tentacles are present already prior to metamorphosis (Wanninger *et al.*, 1999).

Chitinized gill support: Waller (1998) proposed this as a synapomorphy for all Conchifera except Tryblidia. However, basal clades of Gastropoda (Patellogastropoda, Neritimorpha) lack ctenidial skeletal rods. In cephalopods the ctenidial skeleton is situated in the afferent axis, whereas gastropods and bivalves have efferent rods. Therefore homology of the ctenidial skeleton is very improbably and the character is not coded herein.

Position of ctenidia or secondary gills: A posterior (versus lateral and posterior) position of ctenidia or secondary gills has been considered as a synapomorphy for Scaphopoda, Gastropoda, and Cephalopoda by Waller (1998). However, lepidopleuran Polyplacophora and protobranch Bivalvia also have purely posteriorly situated ctenidia, and certain Patellogastropoda (*e. g. Patella*) exhibit also lateral gills. Therefore this character is not coded in this study.

RESULTS

The parsimony algorithm revealed a single most parsimonious tree with 95 steps, CI (excluding uninformative characters) = 0.701; RI = 0.780 and RC = 0.566 (Fig. 2). Various re-arrangements of outgroups always show (1) that Kamptozoa and Mollusca as well as Annelida and Sipuncula are sister taxa. (2) The *Aplacophora* and *Aculifera* appear as basal and paraphyletic Mollusca, with the Solenogastres, Caudofoveata, and Polyplacophora as subsequent offshoots. (3) Thus, the monophyly of Testaria (Polyplacophora and Conchifera), further of Conchifera and Cyrtosoma (or better Visceroconcha: Gastropoda and Cephalopoda) is confirmed.

Coding of character #15 as unordered results in three most parsimonious trees again with 95 steps. Tree #2 of these is identical to that of Fig. 2, the (majority rule and strict) consensus of all most parsimonious trees shows a polytomy: Tryblidia, Bivalvia, [Scaphopoda (Gastropoda & Cephalopoda)].

DISCUSSION

Solenogastres as the first molluscan offshoot and the Hepagastralia-concept

It was not before the recent cladistic study by Salvini-Plawen and Steiner (1996) that anyone regarded the Solenogastres as the earliest molluscan offshoot and considered the monophyly of the remaining classes. Whereas Ivanov (1996), Scheltema (1993, 1996) and Waller (1998) favoured the Aplacophora-concept, also earlier papers by Salvini-Plawen (mainly 1972, 1981, 1985, 1991) argued for Caudofoveata versus the remaining classes (Adenopoda-concept). Despite some differences in character coding (see above), the present result is in full accordance with that of Salvini-Plawen and Steiner (1996). Herein I name the monophyletic clade consisting of Caudofoveata and Testaria as **Hepagastralia**[1] reflecting the main synapomorphy, the highly distinct and complex subdivision of the midgut. A clade Hepagastralia is additionally supported by

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[1] I regard it as nonsense to argue about the rank of Hepagastralia as a "sub- or infraphylum" or as a "mega- or gigaclass."

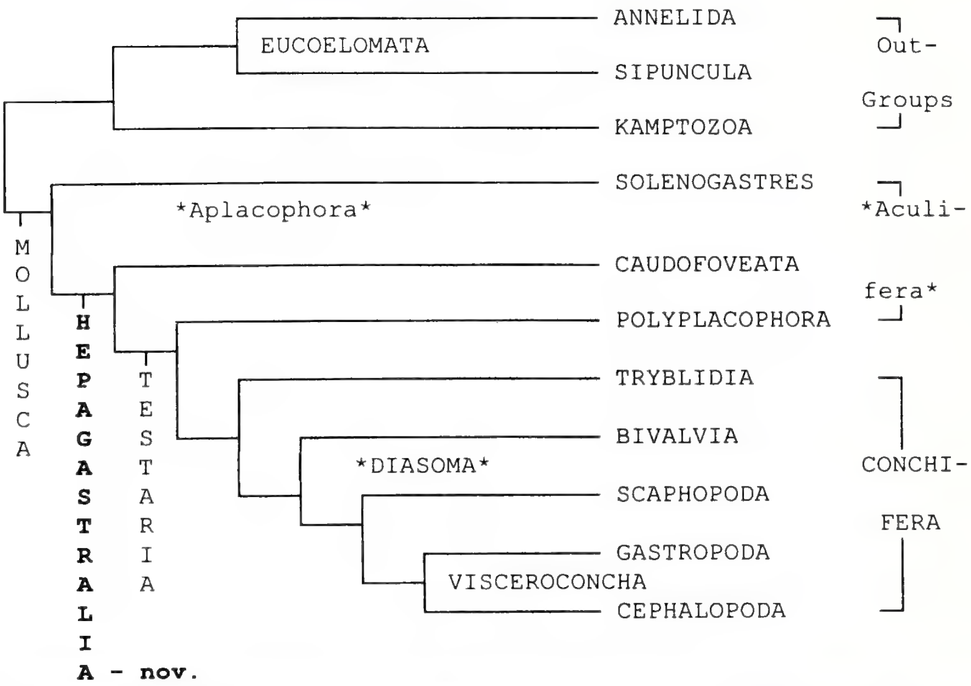

Fig. 2. Single, most parsimonious tree of the current parsimony analysis revealed by coding character #15 as ordered. It is identical to tree #2 (of three), if #15 is coded unordered. *Taxon* means a paraphyletic group.

further characters, the presence of ctenidia, loss of pedal cirri, and presence of a true radular membrane. None of the latter characters is unequivocal, however (see character analysis and below).

Solenogastres show high variability in the principal configuration (anatomy, histology) of the foregut glands and in the number and arrangement of the osphradia (*e .g.* Salvini-Plawen, 1978). Considering the basal position of the Solenogastres in the molluscan framework, one gets the impression that in the Solenogastres these characters are not yet fully constrained, in contrast to the remaining classes. Moreover, the Solenogastres seem to be the only extant molluscan group in which the original, solely ciliary type of locomotion has been retained. In particular the anteriorly placed ciliary "pit", which consists of compound cilia (Haszprunar, 1986) is strikingly similar to the anterior part of the foot sole of nearly all benthic larvae of Kamptozoa (Nielsen, 1971; see also Haszprunar *et al.*, 1995), although fine-structural details are still lacking in the latter case.

Position of Tryblidia

Recent microanatomical and ultrastructural investigations (Haszprunar and Schaefer, 1997a,b; Schaefer and Haszprunar, 1997a,b) provided evidence that the Tryblidia are not "living fossils" or even "Archi-Mollusca". Herein the Tryblidia appear as an early but not necessarily earliest (see above) conchiferan offshoot.

As outlined above the monophyly of the remaining

Character state distribution in Fig. 2.

h = homoplasy, r = reversal
Eucoelomata: #2:0->1; #13:0->1; #20:1->0(h,r); #22:0->1;
NN (Sinusoida = Kamptozoa & Mollusca): ?#20:0->1(r); #24:0->1; #42:0->1;
Mollusca: #3:0->1(r); #7:0->1(r); #8:0->1; #9:x->1; #14:0->1; #23:0->1(r); #25:x->1(r); #26:0->1; #30:0->1; #35:0->1(h); #37:0->1(r); #48:0->1; #51:x->0; #56:0->1(r);
Hepagastralia: #10:0->1(r); ?#20:1->0; #38:0->1; #43:0->1;
Testaria: #4:0->1; #5:0->1; #9:1->0; #12:0->1(h); #15:0->1; ?#20:1->0(h,r); #34:1->0; #39:0->1; #40:0->1; #41:0->1(r); #44:0->1; #46:0->3; #51:0->1; #53:x->0; #57:1->0; #58:0->1(r);
Conchifera: #1:1->0; #3:1->0(r); #4:1->2; #6:0->1; #7:1->0(r); #9:1->0(r); #36:0->1(h,r); #45:0->1(r); #52:0->1; #55:0->1;
NN (Biv, Sca, Gas, Cep): #15:1->2; #50:0->1(r);
NN (Sca, Gas, Cep): #15:2->3; ?#16:0->1(r in Sca and Gas); #45:1->0(r); #47:0->2;
Visceroconcha: #9:0->1(r);#17:0->1; #51:1->2; #53:0->1; #54:0->1(h);
Annelida (Ann): #27:0->2(h); #31:2->4; #36:0->1(h,r);
Sipuncula (Sip): #29:1->0(h); #35:0->1(h); #46:0->1; #47:0->1(h);
Kamptozoa (Kam): #12:0->1(h); #21:1->0; #28:1->0; #33:0->1(h); #47:0->1(h);
Solenogastres (Sol): #19:0->1(h);
Caudofoveata (Cau): #34:0->1(h in Sol); #49:0->1(h in Sol);
Polyplacophora (Pol): #11:0->3; #19:0->1(h); #33:0->1(h);
Tryblidia (Try): #11:0->2; #25:1->0(r); #27:0->2(h); #32:0->1; #46:3->2; #56:1->0(r);
Bivalvia (Biv): #18:0->1(h in Sca); #36:1->0(r); #37:1->0(r); #58:1->0(r);
Scaphopoda (Sca): #10:1->0(r); #15:1->4(h); #23:1->0(r); #31:2->1; #45:1->0(h,r); #56:1->0(r);
Gastropoda (Gas): #15:3->4; #19:0->1(h); #31:2->0; ?#50:1->0(r);
Cephalopoda (Cep): #24:1->2; #29:1->0(h); #33:0->1(h); #35:1->0(r); #41:1->0(r);

conchiferan classes depends on the coding of character #15 (number of shell muscles) clade. In any case there is no unequivocal support for this clade, therefore this clade is not named. The proposed term "Ganglioneura" (Lauterbach, 1983) is in any case inappropriate, since primitive gastropods, bivalves, and cephalopods do not show true ganglia.

Position of Scaphopoda

Applying the Hennigian method Waller (1998) has strongly argued against the Diasoma-concept (Rostrochoncha, Scaphopoda and Bivalvia monophyletic). Instead he favoured a clade "Gastropoda (Scaphopoda and Cephalopoda)." As outlined in the character analysis many of his proposed synapomorphies for this arrangement are not accepted herein. Nevertheless, the present analysis again contradicts the Diasoma-concept and argues for monophyly of Scaphopoda, Gastropoda and Cephalopoda. However, contrary to Waller (1998) the sister-group relationship of Gastropoda and Cephalopoda is very well supported by no less than four non-homoplastic synapomorphies. On the other hand, none of the proposed synapomorphies is unequivocal and all show homoplasies within the terminal taxa. Therefore this clade is not named again.

Implications for the groundplan of the Mollusca

The consideration of the *Aplacophora* as the basic molluscan level of organization has major implications for the understanding and reconstruction of the molluscan stem species (HAM = "Hypothetical Ancestral Mollusc"). Many shared aplacophoran features can now reasonably be considered as characters of HAM (see also Salvini-Plawen, 1972, 1981, 1985, 1991; Haszprunar, 1992; Salvini-Plawen and Steiner, 1996): worm-shaped body with chitinous cuticles covered with aragonitic spicules or scales; distinct body wall musculature; posterior mantle cavity with extrapallial osphradium; distichous radula and carnivory; urinogenital ducts and openings.

Up to now all reconstructions of the molluscan archaetype show ctenidia. The lack of ctenidia in the Solenogastres has been regarded as a secondary loss comparable to those in Scaphopoda and many gastropod taxa, and this scenario is still possible. However, Ockham¥s razor (minimalization of assumptions) favours the assumption of a plesiomorphic lack of ctenidia in the Solenogastres. Indeed, there are many larger species of Solenogastres with respiratory folds in the mantle cavity to increase the respiratory capacity, but none of these gill-leaflets shows the specific ctenidial structure.

Outlook

Is this the final word or the final decision concerning the *Aplacophora*? Of course it is not, since phylogenetics never is a dogma, but is a matter of probabilities depending on the current state of data, character selection, and basic assumptions. In particular ontogenetic data on the aplacophoran taxa are badly needed to clear up certain points such as the possible presence of a pedal sole and gland in larval or early juvenile Caudofoveata.

The same is true in particular for the larval characters of the Kamptozoa, which appear again crucial for improving our understanding of molluscan origins and early evolution. Important questions concern presence or absence of locomotory cirri at the anterior end of the foot sole comparable to Solenogastres or the details of the (tetraneural?) nervous system of the larva.

I do have some personal experience with these highly enigmatic and interesting taxa, but true experts may have a better chance to improve or modify the provided datamatrix based on their long expertise. Nevertheless, it is the argument and the data and not the author who decides the matter. Thus, the rejection of *Aplacophora* as a monophyletic taxon and accordingly the alternative Hepagastralia-concept is the most parsimonious assumption and thus the most probable solution at the present stage of knowledge.

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Considerations on Paleozoic Polyplacophora including the description of *Plasiochiton curiosus* n. gen. and sp.

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Abstract: Numerous occurrences of undescribed Paleozoic polyplacophorans have been located, and new taxa of Silurian, Mississippian, and Permain specimens have been recently described. Problems commonly exist in classifying isolated plates at the species level. Sizes and patterns of aesthetes show some differences in plate microstructure between Paleozoic and modern taxa. Recently proposed classifications indicate differences of opinion concerning relationships and inclusion of non-polyplacophoran taxa. A possible, but incomplete, phylogenetic scheme at the family level is presented. *Plasiochiton curiosus* n. gen. and sp. is described from the Devonian of Pennsylvania.

Key Words: Mollusca, Polyplacophora, Paleozoic, classification, phylogeny

Paleozoic polyplacophorans are not as rare stratigraphically and numerically as one might be led to believe from the published literature, largely as a result of collections remaining undescribed. Collections by the author and associates in Pennsylvanian age strata of the Appalachian basin have found over 50 localities containing one to hundreds of plates of polyplacophorans. In many cases, specimens are placed in repositories with little or no reference to their existence. Inquiries to museums, universities, and individuals commonly turn up specimens, many of which are important in our understanding of the classificiation and phylogeny of the class. It is likely that individuals processing samples for ostracodes and foraminifers, have found, but did not recognize polyplacophoran plates, especially if the plates were fragmented. It is important to illustrate and specify in the literature the location of specimens to make known their existence.

Illustrated specimens herein are in the repositories of the National Museum of Natural History (USNM), Ohio University Zoological Collections (OUZC), and New York State Museum (NYSM).

RECENT STUDIES

Since I began work on this study a number of undescribed specimens have been found. These include: two Ordovician plates of *Gotlandochiton* from Minnesota and two plates of *Chelodes* from the Ordovician of Alabama (Fig. 1); Silurian specimens from California containing new species of *Paleochiton* and *Thairoplax*, only the second known Silurian fauna from North America (Hoare, 2000a); an articulated partial specimen of a new genus from the Devonian of Pennsylvania (described herein); Devonian plates of *Arcochiton*? and *Gotlandochiton* and Mississippian plates of *Gryphochiton* from Australia (Hoare and Cook, 2000); an abundant Mississippian fauna from Iowa containing new species of *Gryphochiton* and *Euleptochiton* and three new genera (Hoare, 2000c), a fauna quite different from that of the Salem Limestone in Indiana described by Kues (1978); two Mississippian plates of *Gryphochiton* from Kentucky, an undetermined Mississippian plate from Morocco, and a Permian plate of *Cymatochiton* from Sicily (Fig. 2); and Permian specimens from Argentina (Hoare and Sabattini, 2000), Malaysia (Hoare, 2000b), and Oregon (Hanger *et al.*, 2000), containing a number of new genera and species. A new Devonian genus from Germany and a new Permian genus from the U. S. are in press (Hoare, 2001). The Malaysian specimens are particulary interesting in their very large size with thick shell material. Recent studies by Cherns (1998a, b) have added significantly to our knowledge of Silurian polyplacophorans. Undoubtedly there are still numerous taxa that have not been found or described.

A significant collection of Lower Carboniferous specimens from Belgium has also been discovered. A study of this collection plus known collections at Harvard University and the Smithsonian Institution will allow a

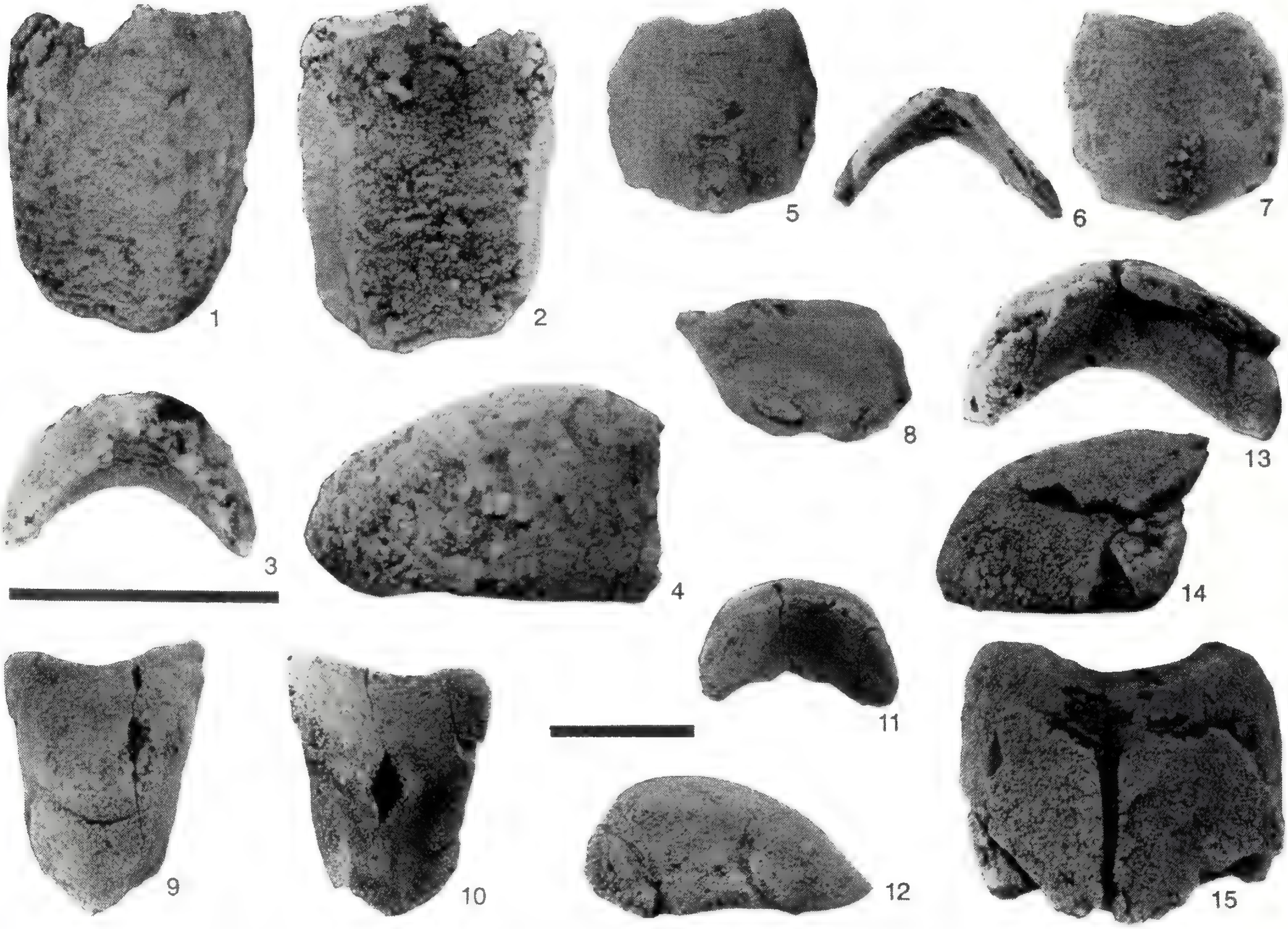

Fig. 1. 1-8, *Gotlandochiton* sp., Oneata Fm. (Ordovician), Minnesota. 1-4, dorsal, ventral, anterior, and right lateral views of a tail plate, USNM 501824 (bar scale = 1 cm); 5-8, dorsal, posterior, ventral, and right lateral views of an intermediate plate, USNM 501825. 9-15, *Chelodes* sp., Odenville Fm. (Ordovician), Alabama. 9-12, dorsal, ventral, anterior, and left lateral views of a tail plate, USNM 501826; 13-15, posterior, right lateral, and dorsal views of an intermediate plate, USNM 501827 (bar scale = 1 cm).

review of their taxonomic relationships which, as described and illustrated by de Koninck (1842, 1883), Münster (1839, 1843), and de Ryckholt (1845, 1852), appear to contain a number of synonymous taxa.

PROBLEMS

The study of fossil polyplacophorans is challenging because the dorsal plates usually become disarticulated and are most commonly found as isolated entities. Complete articulated specimens are rare, although the occurrence of numerous specimens of *Glaphurochiton concinnus* (Richardson, 1956) in the Pennsylvanian Francis Creek Shale in Illinois is a notable exception. These specimens also show the presence of radula, girdle, and spines (Yochelson and Richardson, 1979). Incomplete articulated specimens are also helpful in determining the correct association of isolated plates.

Isolated plates, when described and named, have led to taxonomic confusion. As an example, Dunlop (1922), by careful collecting at one locality, was able to show that *Chiton cordatus* Kirkby, 1859, a head plate, *C. armstrongianus* Etheridge, 1882, an intermediate plate, and *C. gemmatus* Kirkby, 1862, a tail plate, were all parts of the same species, *C. cordatus* Kirkby, 1859 [=*Lekiskochiton cordatus* (Kirkby, 1859)]. Because intermediate plates are usually more commonly found, a number of species have been erected on the basis of a single plate. Tail plates of most Paleozoic taxa are more diagnostic than head or intermediate plates and distinction between genera and species can often be made on these plates alone. Tail plates also provide much of the ontogenetic evidence of a taxon (Fig. 3).

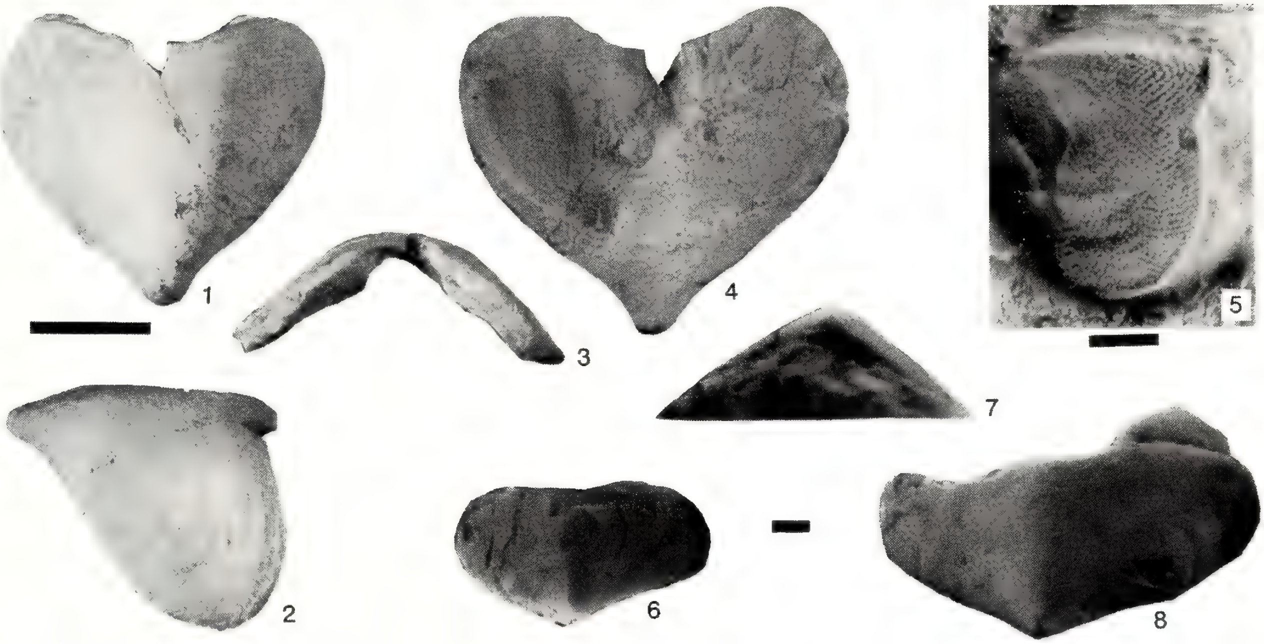

Fig. 2. 1-4, *Cymatochiton* sp., Socio Ls. (Permian), Sicily. Dorsal, right lateral, anterior, and ventral views of an intermediate plate, USNM acc. No. 179,820 (bar scale = 1 cm). Note large apical area on ventral surface. 5, Undetermined sp., Mississippian formation, Morocco. Oblique right lateral view of an intermediate plate, OUZC 1633 (bar scale = 1 mm). 6-8, *Gryphochiton parvus* (Stevens), Tribune Ls. (Mississippian), Ky. Dorsal and posterior views of an intermediate plate, USNM 508371, and a dorsal view of an intermediate plate, USNM 508372, (bar scale = 1 mm).

PLATE MICROSTRUCTURE

Previous studies of plate microstructure (*e. g.* von Knorre, 1925; Bergenhayn, 1930; Haas, 1972) have normally been made by cutting the plates vertically showing disposition of shell layers and the occasional aesthete exposed in the section. Such sections do not show the pattern of the aesthetes. In cutting a piece of a plate parallel to and just below the dorsal surface of the tegmentum the size, pattern, and distribution of the aesthetes can be determined. Haas (1972) cut Recent material parallel to the plate surface.

Specimens of nine species representing six genera of Mississippian and Pennsylvanian taxa were sectioned. Preservation of the aesthetes ranged from poor to excellent (Fig. 4) and measurements of diameters and spacing were made. Several preliminary conclusions were reached:

1. No size distinction for micraesthetes and megaesthetes was present in any specimen;
2. No pattern of micraesthetes encircling megaesthetes or any other circular pattern was seen. Such patterns must have developed post-Paleozoic;
3. For coarser surface ornamentation of pustules, the diameter of the aesthetes was larger, with one aesthete per pustule;
4. Aesthete diameter ranged in size from 19.3 µm to 31.2 µm in the specimens;
5. Several specimens showed aesthetes centered in polygonal structures. Whether this is an artifact of preservation or a structure related to plate development is unknown.

CLASSIFICATION

A number of multiplated organisms other than polyplacophorans are known to occur in the Paleozoic. The opportunity to study specimens of several of these taxa has led the author to reevaluate what should be or should not be included in the Polyplacophora. Some of these (*e. g. Diadeloplax, Strobilepis, Aenigmatectus*) from the upper Paleozoic have some characteristics in common with polyplacophorans including mucros on tail plates, insertion plates, and apical areas. However, the shapes, arrangements, and ornamentation of the plates are usually quite different and the canal system in the plates is different from the aesthetes in polyplacophorans (Hoare and Mapes, 1995, 1996).

Bischoff (1981) included Ordovician-Silurian phosphatic multiplated organisms from Australia in the Polyplacophora. Besides being of a different composition these plates show structural differences from polyplacophorans such as plate growth patterns, microstructure, and supposed insertion plates. Yu (1984, 1987) has illustrated and described a number of multiplated taxa from the

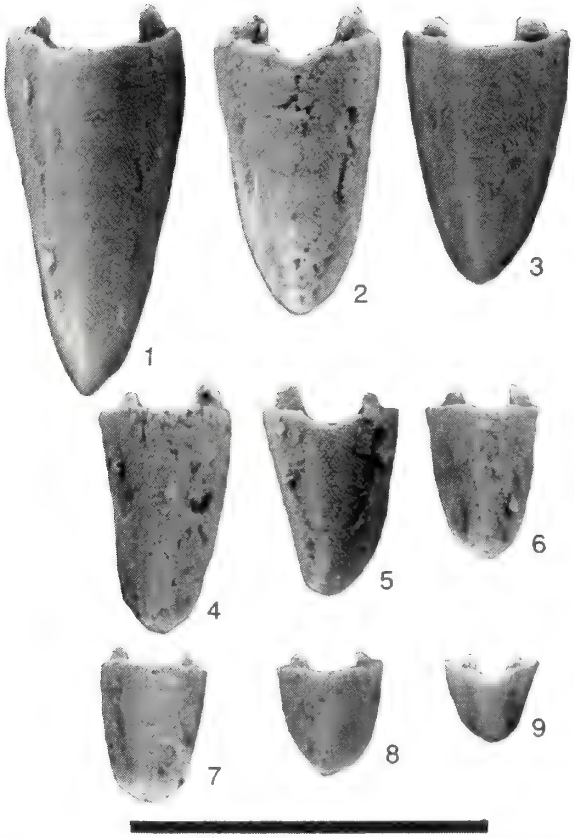

Fig. 3. *Acutichiton allynsmithi* Hoare, Mapes, and Atwater. Gene Autry Fm. (Pennsylvanian), Oklahoma. 1-9, dorsal views of a series of tail plates showing increase in size and change in shape during ontogeny, OUZC 1621-1629 (bar scale = 0.5 cm).

Lower Cambrian of China placing them in the Polyplacophora under five new families. These are small specimens, mostly less than 0.5 mm in width. Sirenko (1997) questioned the inclusion of these in the Polyplacophora, a conclusion with which I agree.

The family Septemchitonidae Bergenhayn, 1955, contains multiplated organisms that developed plates that curve inward ventrally almost closing off the area for the position of the foot as illustrated by Dzik (1994, fig. 29). These plates are totally unlike polyplacophoran plate shape and the family is here omitted from the class. It is expected that many other types of multiplated organisms will eventually be discovered in Paleozoic strata.

Most recently, Sirenko's (1997) classification of polyplacophorans recognized four orders, five suborders, and 14 families, including one new family, in the Paleozoic. Changes from his scheme suggested here (Fig. 5) include the recognition of the family Matthevidae Walcott (1886) in place of Chelodidae Bergenhayn (1943), the elimination of the family Septemchitonidae Bergenhayn (1955), and the addition of the family Choriplacidae Cotton and Weeding (1939). The latter is included based upon the new Devonian genus and species described herein, which has many characters comparable to the genus *Choriplax* Pilsbry, 1894.

PHYLOGENY

As with the classification, there are more questions than answers related to the phylogeny of the Polyplacophora at this time. This situation is true even to the extent of determining the origin of the class and relationship with other mollusks. It is not the purpose of this study to discuss the origin of the phylum or that of the polyplacophorans. However, polyplacophorans are so different from other mollusks in terms of their skeletal and anatomical structures that it seems likely that the phylum is polyphyletic.

Yu (1989) proposed a multiplated ancestral stem leading to the polyplacophorans from which other molluscan classes developed. The Polyplacophora undoubtedly developed from a segmented ancestral form but it seems very unlikely that the single or double plated classes of other mollusks arose from a similar ancestor (Runnegar and Pojeta, 1974; Pojeta, 1980).

Figure 5 shows a possible phylogenetic relationship at the family level. It is based in part on the classification given by Sirenko (1997). There will be additional families added to this scheme based upon the studies of Cherns (1998a, b) in the Silurian, possibly in the Permian by the author, and by the discovery of additional specimens in the future.

SYSTEMATICS

Class Polyplacophora de Blainville, 1816
Order Neoloricata Bergenhayn, 1955
Suborder Lepidopleurina Thiele, 1910
Family Choriplacidae Cotton and Weeding, 1939
Genus *Plasiochiton* n. gen.

Type species. *Plasiochiton curiosus* n. sp.

Diagnosis. Intermediate plates subrectangular, wider than long, moderately arched, broadly convex; semicircular raised areas marked by concentric ridges on anterior jugal areas.

Description. See under *Plasiochiton curiosus* n. sp.

Discussion. This taxon is based upon a single incomplete articulated specimen. *Plasiochiton* differs from *Glyptochiton* de Koninck, 1883, in the shape of the plates and the shape and position of the raised jugal portion. The Recent genus *Choriplax* Pilsbry, 1894, is similar to *Plasiochiton* in its subrectangular plates with a raised and restricted tegmentum region located on the median portion

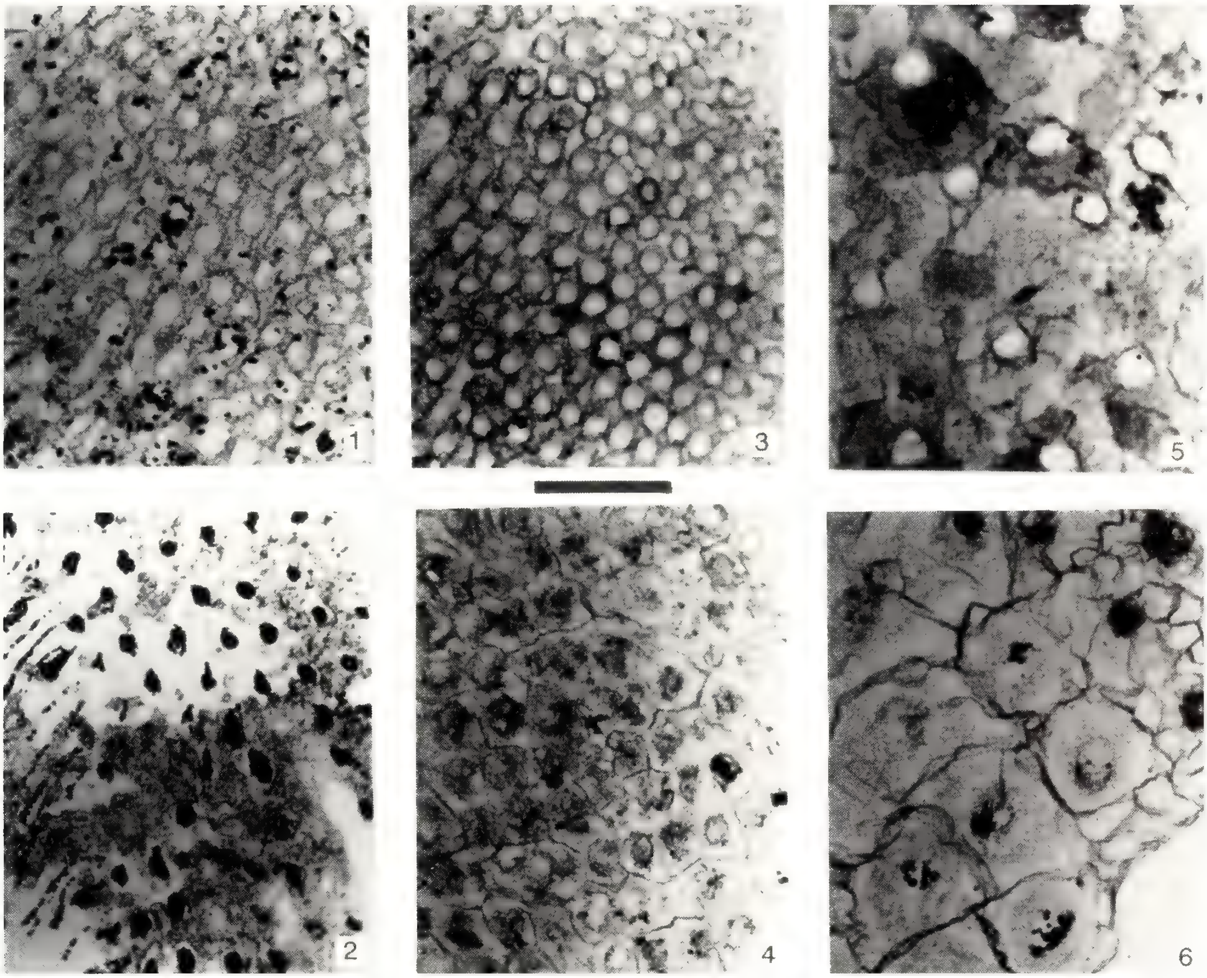

Fig. 4. Thin sections showing size and distribution of aesthetes on intermediate plates. 1, *Acutichiton pyrmidalus* Hoare, Sturgeon, and Hoare, Vanport Ls., Ohio; 2, *Elachychiton juxaterminus* Hoare and Mapes, Imo Fm., Ark.; 3, *Euleptochiton spatulatus* (Hoare, Sturgeon, and Hoare), Vanport Ls., Ohio; 4, *Gryphochiton simplex* (Raymond), Vanport Ls., Ohio; 5-6, *Glaphurochiton carbonarius* (Stevens), Putnam Hill Sh. and Washingtonville Sh., Ohio, OUZC 1633-1638 (bar scale = 100 μm). Note the differences in size and distribution of the aesthetes and the polygonal structure in 4 and 6. (Sections prepared by B. R. Hoare).

of the intermediate plates. *Plasiochiton* is questionably assigned to the family Choriplacidae on this basis.

Occurrence. Known only from the type locality of *P. curiosus*, Devonian (Erian).

Etymology. Greek, *plasion*, oblong body.

Plasiochiton curiosus n. sp.

Fig. 6

Polyplacophoran Petzold, Clark, Makin, Owens, and Perry, 1992, p. 342, Fig. 1.

Diagnosis. As for the genus.

Description. Incomplete, articulated specimen of five plates preserved as mold of ventral surface; intermediate plates subrectangular, moderately arched, broadly convex, wider than long; posterior margins straight meeting lateral margins at right angles; anterior margin not observed; raised anterior jugal areas marked by concentric ridges in U-shaped pattern; raised area smaller towards posterior of specimen; shell material thin; head and tail plates unknown.

Measurements. Size of exposed specimen 17 mm long, 4 mm wide; exposed plates range from 1.8 to 2.5 mm in length and from 4.0 to 4.6 mm in width; estimated approximate total length 30 mm, total width 9.0 mm, and total height 4.0 mm.

Etymology. Latin, *curiosus*, odd, strange.

Type. Holotype, USNM 456242.

Type locality. Devonian Sherman Ridge Member of the Mahantango Formation exposed in an abandoned shale pit at the intersection of U.S. 11-15 and Pennsylvania 104, Perry Co., Pennsylvania, 40°36'52"N, 76°57'21"W, Millersburg 7.5 minute quadrangle.

Discussion. Devonian polyplacophorans are little known in

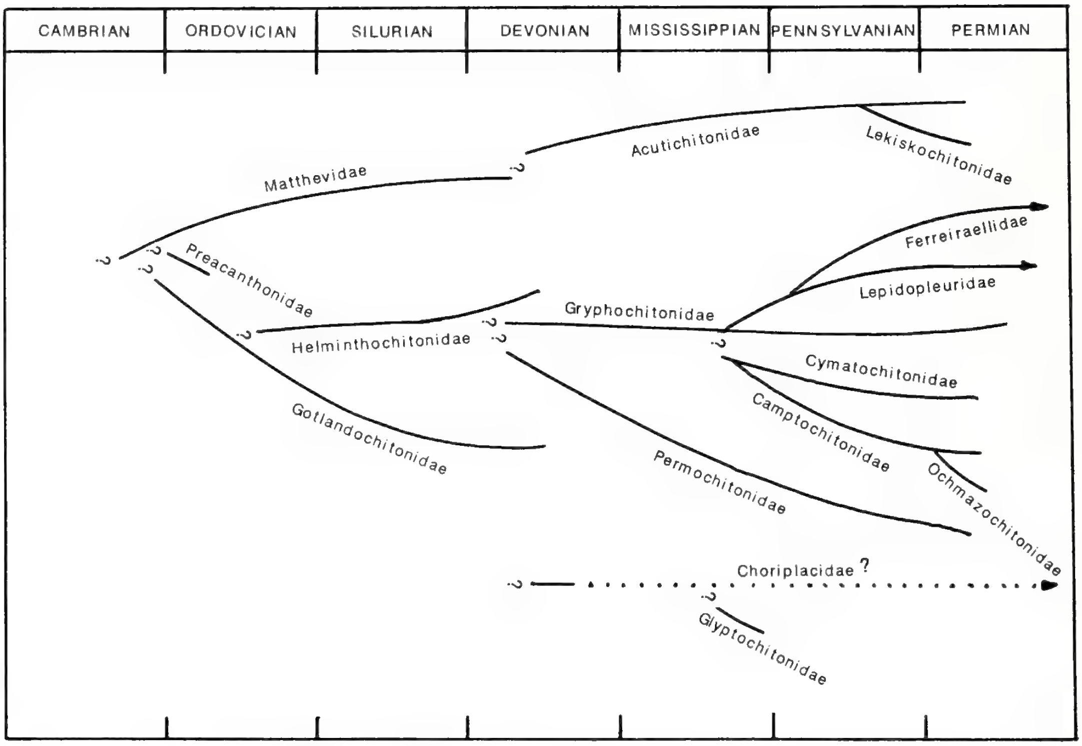
CAMBRIAN
ORDOVICIAN
SILURIAN
DEVONIAN
MISSISSIPPIAN
PENNSYLVANIAN
PERMIAN
Matthevidae
Acutichitonidae
Lekiskochitonidae
Ferreiraellidae
Lepidopleuridae
Preacanthonidae
Gryphochitonidae
Helminthochitonidae
Cymatochitonidae
Gotlandochitonidae
Camptochitonidae
Permochitonidae
Ochmazochitonidae
Choriplacidae ?
Glyptochitonidae

Fig. 5. A schematic phylogenetic diagram showing possible relationships at the family level.

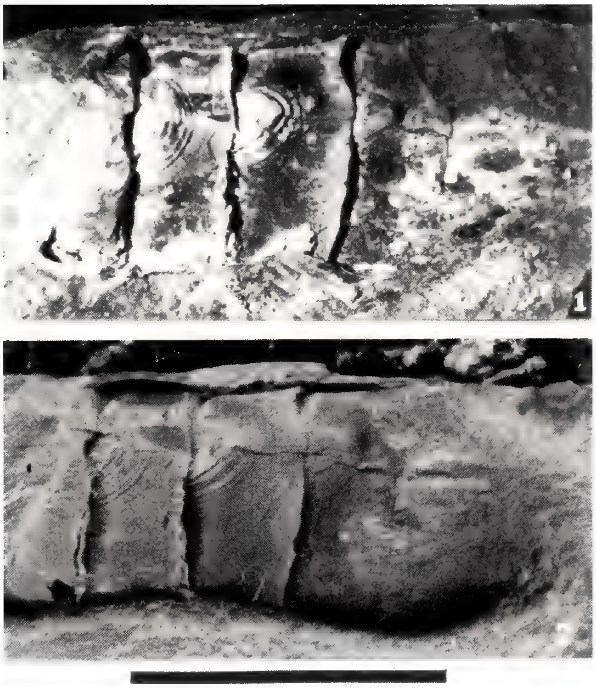

Fig. 6. *Plasiochiton curiosus* n. gen. and sp. 1, uncoated and 2, coated dorsally oblique views of a partial articulated ventral mold, Mahantango Fm. (Devonian), Pennsylvania, USNM 456242 (bar scale = 1 cm). Note the subrectangular shape of the plates and the medial anterior restriction of the tegmentum layer. (Fig. 1 from Petzold *et al.*, 1992, p. 342, published with the permission of the publisher and author).

North America and this specimen is important to our understanding of the development of the class in the Paleozoic. Unfortunately the shell material is lacking, but the mold is diagnostic in the shape of the plates and the semicircular structure on the anterior portion of the jugal area. In general the raised structure is similar to that of *Glyptochiton* de Koninck, 1883, from the Lower Carboniferous of Belgium and the United Kingdom but differs in position on the plates and the shape of the plates of the latter is different. The complete size and shape of the plates of *Plasiochiton curiosus* cannot be determined without destroying a portion of the specimen.

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Cambrian monoplacophoran molluscs (Class Helcionelloida)

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Abstract: Cambrian monoplacophoran molluscs (Class Helcionelloida) are morphologically diverse, but also show considerable interspecific variation. While usually small (1-2 mm), they may achieve a size of 30 mm or more. Shell form ranges from low limpets to high cones and these may be straight or curved; coiled shells may be bilaterally symmetrical, dextral or sinistral. This diversity provides a fertile ground for speculation about early molluscan evolution and almost all extant classes of Mollusca have been recognised here. Certainly, molluscs can be traced back to the late Vendian (late Precambrian), but not all classes appeared during the Vendian-Cambrian transition. Small-sized late Vendian-Middle Cambrian molluscs (helcionelloids) show little resemblance to crown molluscan groups, including the living monoplacophorans (tryblidiid Tergomya).

Key Words: Cambrian, Mollusca, monoplacophoran, Helcionelloida, evolution

Most Cambrian monoplacophoran molluscs are morphologically far removed from the familiar present day tryblidiid tergomyan *Neopilina* Lemche, 1957 or its Early Palaeozoic antecedents *Pilina* Koken, 1925 and *Tryblidium* Lindström, 1880. Placed in the Class Helcionelloida, these Cambrian univalves display a variety of shell forms, bearing witness to the first great diversification of molluscs near the Precambrian-Cambrian boundary (Peel, 1991a, b). Indeed, helcionelloids are represented in the first shelly faunas appearing in the late Precambrian in Siberia (Khomentovsky *et al.*, 1990). The helcionelloid shell is typically bilaterally symmetrical (isostrophic), rapidly expanding and coiled through about one whorl, but limpet-like shells, tall cones and strongly laterally compressed morphologies are also conspicuous. *Aldanella* Vostokova, 1962 is a multi-whorled anisostrophic helcionelloid from the earliest Cambrian, seductively reminiscent of the gastropods that first appeared in the latest Cambrian. Anisostrophic shells of *Pelagiella* Matthew, 1895 can be conspicuous throughout the Cambrian and are conveniently placed within the Helcionelloida, even if their precise relationship remains uncertain.

Helcionelloids may be abundant in strata of Early and Middle Cambrian age. A variety of problematic molluscs are known from the Late Cambrian (*e. g.*, Webers *et al.*, 1992) but the helcionelloid record is uncertain. However, we here report undoubted helcionelloids from the latest Cambrian to early Ordovician of Kazakhstan, representing the youngest known members of the class. Thus, the geological record of helcionelloids overlaps with that of the earliest tryblidiid tergomyans such as *Proplina* Kobayashi, 1933 and most other molluscan classes.

Helcionelloids have been interpreted as torted (gastropods, *e. g.* Knight *et al.*, 1960) and un-torted (monoplacophorans) and this latter hypothesis is currently widely supported (Runnegar and Jell, 1976; Peel, 1991a, b; Geyer, 1994; Runnegar, 1996). Their relationship to tergomyans such as *Neopilina* is disputed, with some authors (Runnegar, 1996 and previous works cited therein) uniting all these untorted univalves in a single Class Monoplacophora. Peel (1991a, b; see also Geyer, 1994) reconstructed helcionelloids with the apex at the posterior and the shell expanding anteriorly (endogastric), as distinct from the anterior apex and posteriorly expanding (exogastric) shell of *Neopilina* and other tergomyans. Following the recommendation of Wingstrand (1985) and others, Peel (1991a, b) abandoned Monoplacophora as a formal taxon, employing Class Helcionelloida for the endogastric branch and Class Tergomya for the exogastric trybilidiid stock. Recognition of helcionelloids as the molluscan ancestral group focuses attention in the search for intermediate forms to molluscan classes such as Tergomya, Gastropoda and Cephalopoda to the Late Cambrian. In contrast, the Rostroconchia, as a direct descendant of the Helcionelloida, and the Bivalvia appear to be represented already in the Early Cambrian (Runnegar and Pojeta, 1985).

THE OLDEST MOLLUSCS

Helcionelloids are present in the oldest shelly faunas from Siberia of late Vendian (Nemakit-Daldynian) age

(Khomentovsky *et al.*, 1990; Gubanov and Peel, 1999). These, and the slightly younger, earliest Cambrian, species *Oelandiella korobkovi* Vostokova, 1962 have been referred to the genus *Latouchella* Cobbold, 1921 by most authors, following the unexplained synonymisation of *Oelandiella* Vostokova, 1962 with *Latouchella* by Missarzhevsky (*in* Rozanov *et al.*, 1969; translated into English as Raaben, 1981). Recent re-description of the type materials of both genera by Gubanov and Peel (1998, 1999) has demonstrated that *Oelandiella* is a valid and recognisable genus whereas the widely reported *Latouchella* is uncommon. *Oelandiella* is characterised by comarginal rugae that cross the dorsum whereas the median dorsal area in *Latouchella* is smooth with rugae restricted to the lateral areas. *Latouchella* has also been confused with *Oelandia* Westergård, 1936 from the Middle Cambrian of Sweden, China and Bohemia. The latter genus, however, is not bilaterally symmetrical since the lateral rugae interdigitate at the dorsum (Peel and Yochelson, 1987; Gubanov and Peel, 1998).

Inflated and strongly rugose shells of *Oelandiella korobkovi* occur contemporaneously with the laterally compressed and generally smooth-shelled *Anabarella* Vostokova, 1962, also originally described from northern Siberia. The close relationship between these two genera is demonstrated by the recent description of rugose *Anabarella* from Spain (Vidal *et al.*, 1999). Recently, we have also identified *Anabarella* from the Lontova Formation (Early Cambrian) of Estonia where it occurs together with *Aldanella kunda* (Öpik, 1926) described by Posti (1978).

SIZE, SYMMETRY, AND VARIATION

Most described Cambrian helcionelloids are small, generally 1-2 mm in length (Runnegar and Pojeta, 1985). This reported size range sometimes reflects environmental, preservational and preparational biases because recovery of material suitable for description is commonly accomplished by digestion of phosphatised carbonate rocks in weak acids. Even in the Tommotian (earliest Cambrian), however, there are helcionelloids more than 30 mm long (Dzik, 1991 and personnal observations) (Fig. 1) while undescribed late Early Cambrian material from Sweden can be of similar size. The taxonomic significance of the size differences is uncertain but the importance of considering size in functional morphological interpretations of early molluscs was stressed by Peel (1991b). An unfulfilled need for ontogenetic studies is also apparent in the taxonomy of helcionelloids.

Archaeospira Yu, 1979 from the earliest Cambrian of China closely resembles *Oelandiella korobkovi* (Fig. 2) and the latter name has been applied by earlier authors to material included within the synonymy of the type species *Archaeospira ornata* Yu, 1979 in the detailed re-description given by Qian and Bengtson (1989). They pointed out that *O. korobkovi* is not known to be asymmetrically coiled while the shell of *A. ornata* is slightly sinistral. Furthermore, they commented that a second Chinese species, *Hubeispira nitida* Yu, 1981, is dextral. Gubanov and Peel (1999) noted that some specimens of *O. korobkovi* were slightly dextral but recent study of material from the earliest Cambrian (Tommotian) of the Selinde River, southern Siberia, demonstrates the presence of sinistral, dextral and planispiral bilaterally symmetrical morphs in the same sample of *O. korobkovi*. The sinistral *Archaeospira* may thus prove to be a synonym of *Oelandiella*. The same samples also show dextral morphs of *O. korobkovi* that approach the morphology of the anisostrophic *Aldanella* (Fig. 3). *Aldanella* itself also shows a similar spectrum of

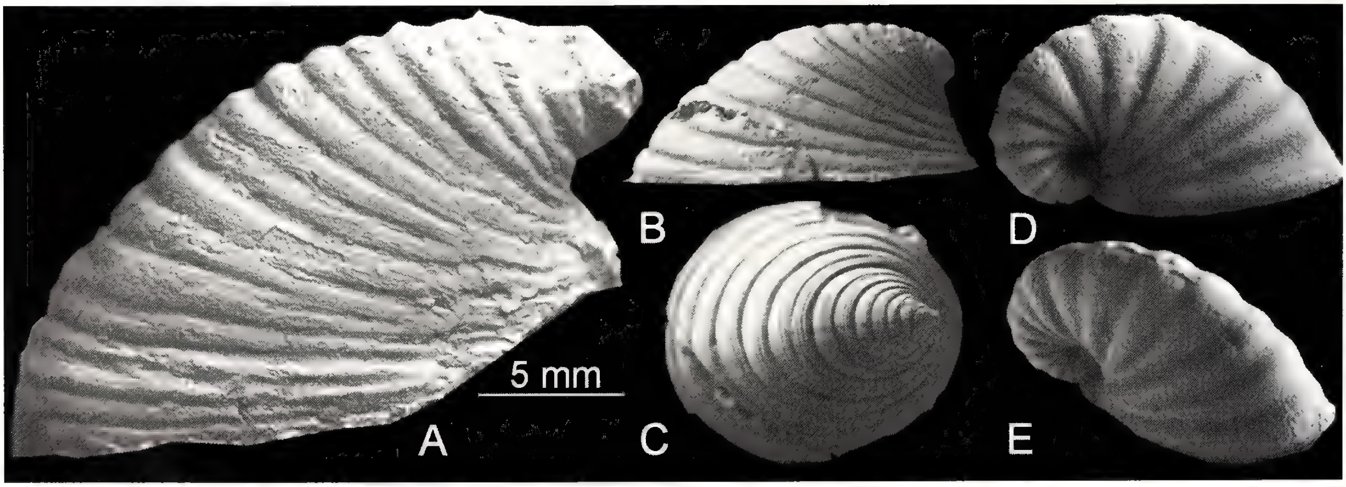

Fig. 1. Large limpet-shaped (A-C) and coiled (D, E) helcionelloid molluscs from the Lower Cambrian (Tommotian, *Dokidocyathus regularis* Zone) of the middle Lena River (southeastern Siberia). **A,** Institute of Palaeobiology, Polish Academy of Sciences, Warsaw (IPB PAN) Ga VIII-3, the largest specimen with broken apex, lateral view. **B, C,** IPB PAN Ga VIII-13, example with well-preserved shell, lateral (B) and apical (C) views. **D, E,** IPB PAN Ga VIII-7, tightly coiled mollusc, lateral (D) and anterio-lateral (E) views.

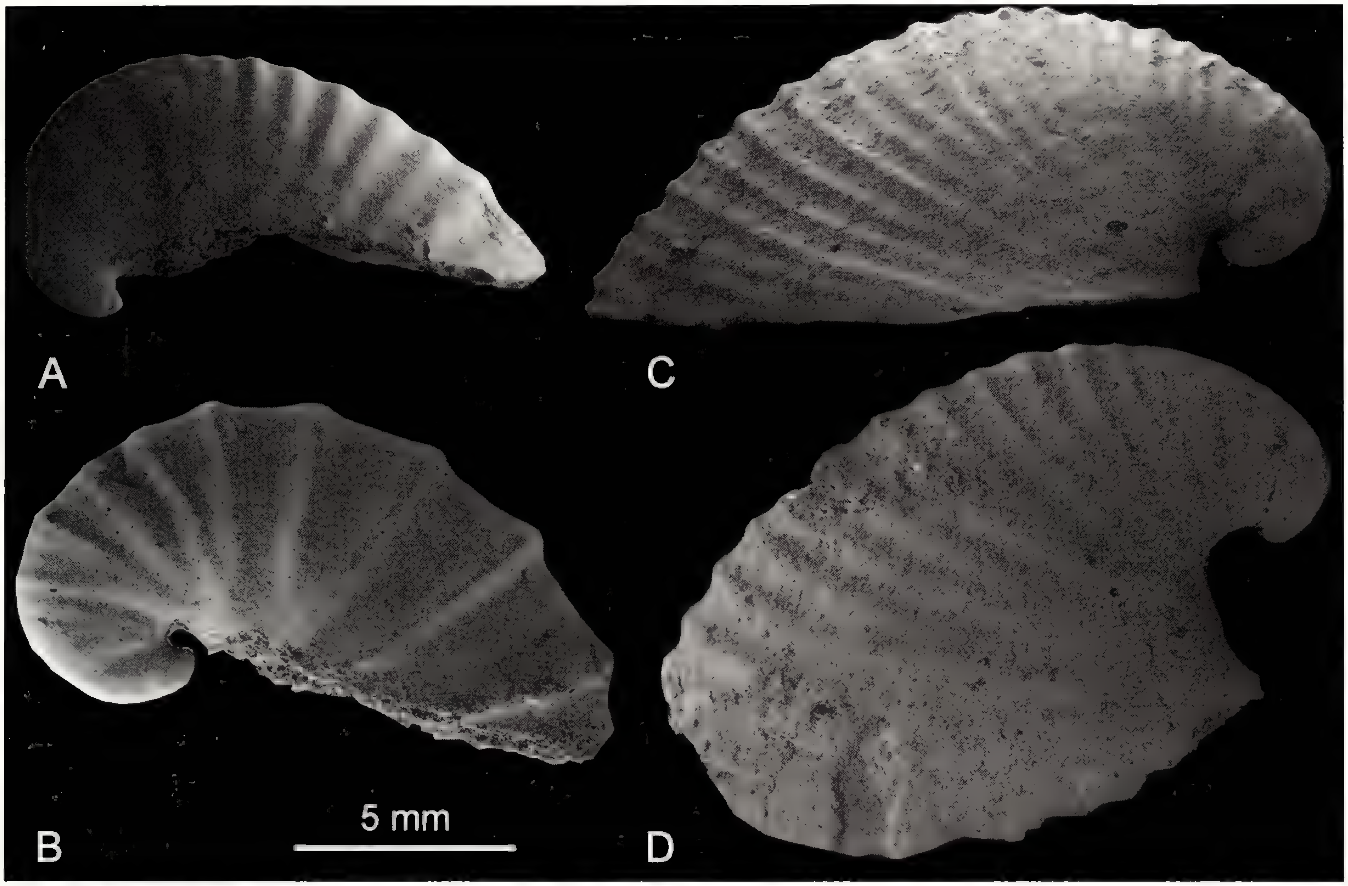

Fig. 2. *Oelandiella korobkovi* Vostokova, 1962 showing variation in comarginal ornamentation and rate of expansion. **A, B,** Museum of Evolution (Palaeontology), Uppsala University (PMU) SIB 1001, 1002, Pestrosvet Formation (early Tommotian) of the Dzhanda River, southeastern Siberia **C, D,** PMU CH 1, 2, Dahai Member (Meisuchunian) of southern China.

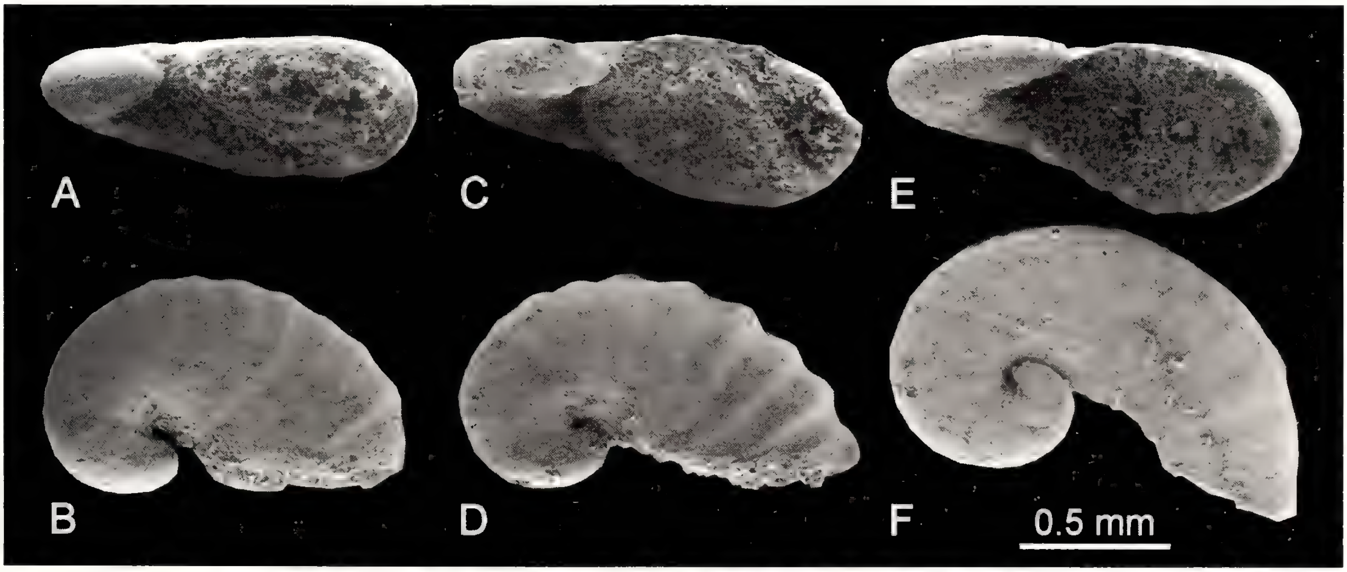

Fig. 3. *Oelandiella korobkovi* Vostokova, 1962 from the lower Cambrian (Tommotian) of the Selinde River, southeastern Siberia. **A, B,** PMU SIB 1003, isostrophic morph in apertural (A) and lateral (apical) (B) views. **C, D,** PMU SIB 1004, dextral morph with comarginal ribs, apertural (C) and apical (D) views. **E, F,** PMU SIB 1005, dextral *Aldanella*-like morph with smooth shell surface, apertural (E) and apical (F) views.

variation occurring within the same sample, and with substantial variation in such characters as rate of whorl expansion, rate of translation along the axis of coiling, tumidity, and tightness of coiling (Fig. 4).

While early Cambrian helcionelloids, including *Aldanella*, appear to be taxonomically diverse, it is now evident from this substantial variation within samples that considerable taxonomic inflation has occurred. Recognition of this variation strengthens interpretation of *Aldanella* as an anisostrophic helcionelloid, refuting suggestions that it could be a gastropod (Runnegar and Pojeta, 1985).

DIVERSITY

Helcionelloids show a remarkable diversity in the Early and Middle Cambrian. The wide aperture and strong comarginal ornamentation suggest that *Oelandiella* was a benthic micro-herbivore or deposit feeder, a mode of life inferred in a variety of other taxa, such as *Latouchella*. Two adaptive morphological trends are clearly visible already in the Tommotian. Expansion of the shell to produce a low cone with a broad to near equidimensional aperture in plan view characterises *Bemella* Missarzhevsky, 1969 and *Helcionella* Grabau and Shimer, 1909, interpreted as benthic deposit feeders adapting to a hardening substratum (Linsley, 1977; Gubanov, 1984). Loss of ornamentation and strong lateral compression in *Anabarella* indicate adaptation to a softer substrate and a semi-infaunal life. This trend from *Oelandiella* to *Anabarella* is continued into *Watsonella* Grabau, 1900 (= *Heraultipegma* Runnegar and Pojeta, 1976), considered to be the oldest member of the Class Rostroconchia Runnegar and Pojeta, 1974 (Pojeta and

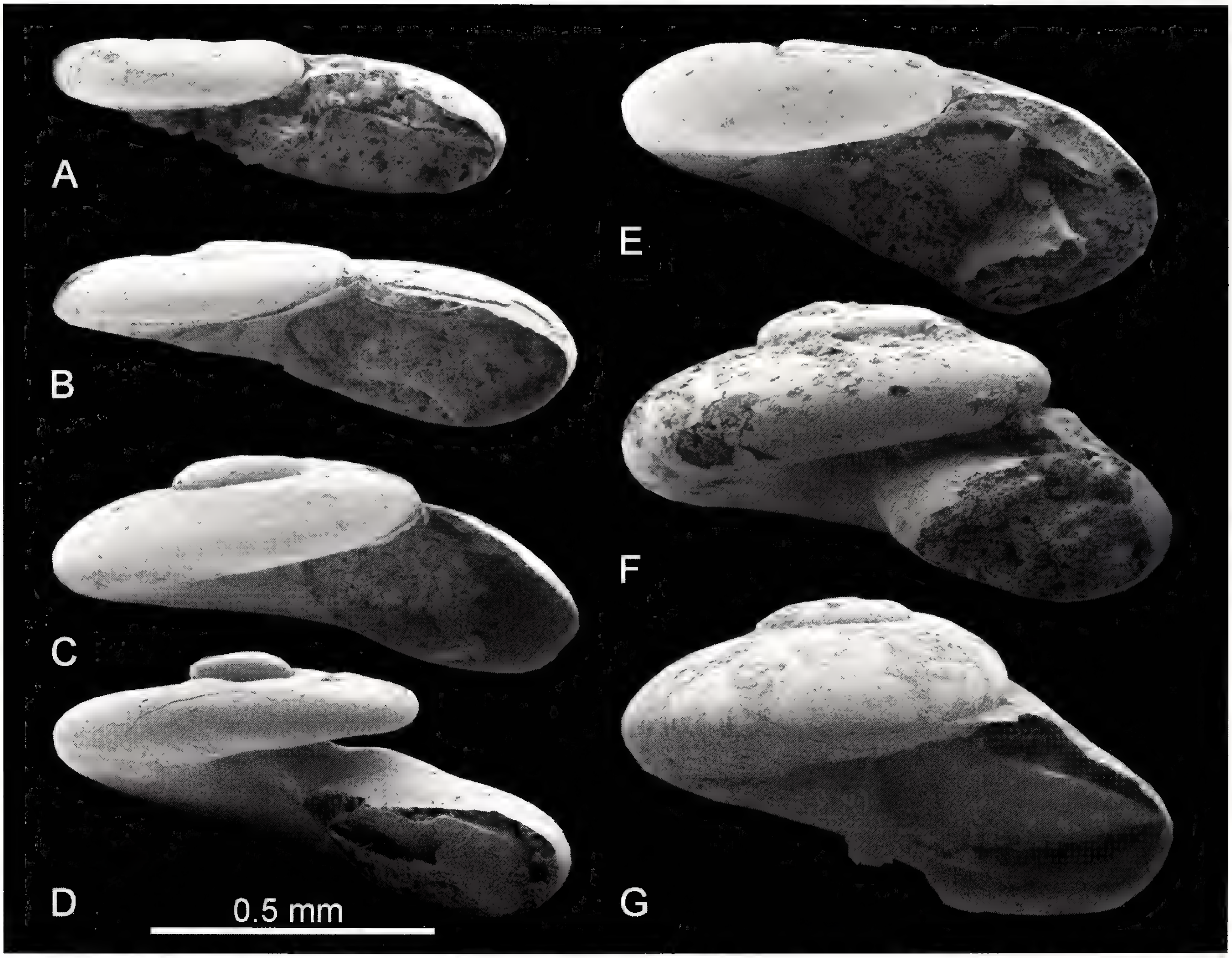

Fig. 4. *Aldanella* sp. from the Lower Cambrian (early Tommotian) of the Anabar River, northeastern Siberia showing the spectrum of shell form variation, PMU SIB 1006-1012, apertural views of all specimens.

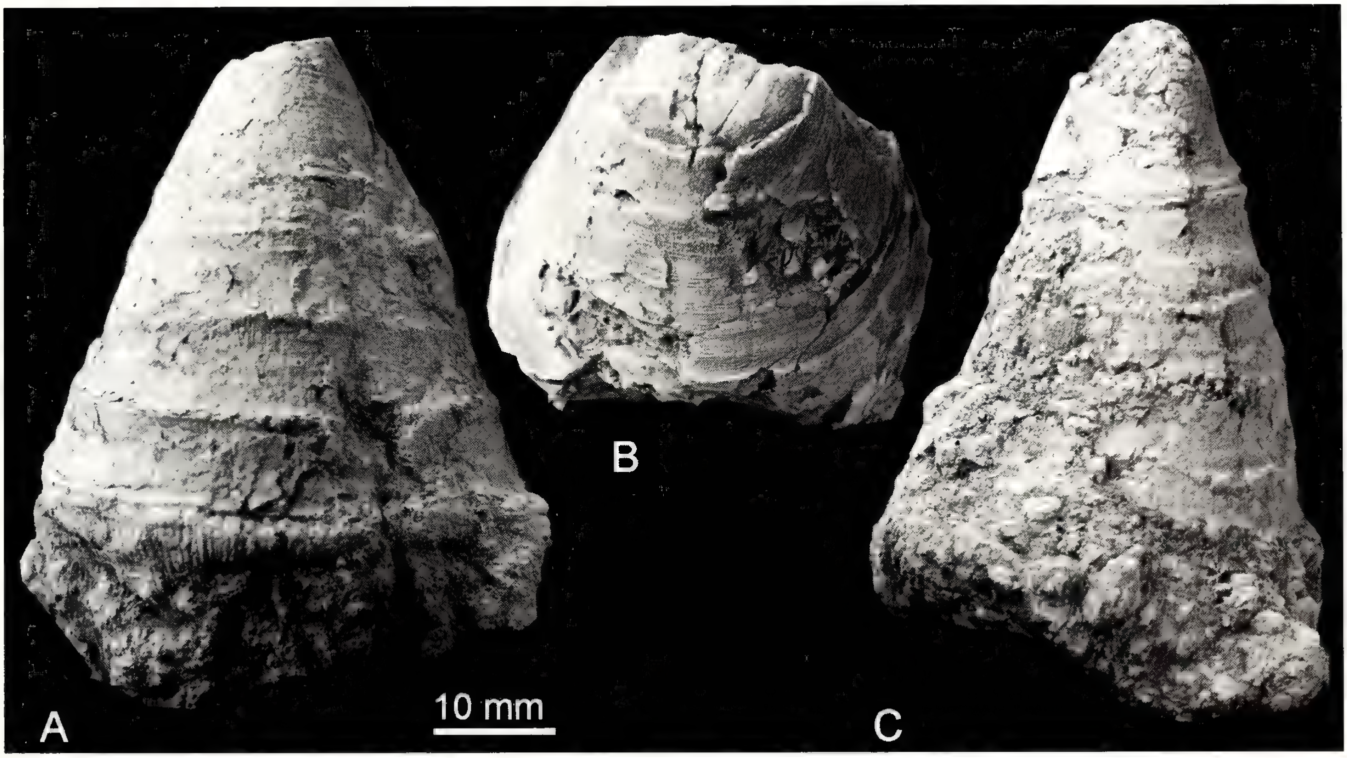

Fig. 5. The hydrothermal vent mollusc *Thermoconus shadlunae* Little, Maslennikov, Morris, and Gubanov, 2000, from the Silurian volcanic rocks of Yaman-Kasy, southern Urals, Russia. **A, C,** lateral views of two specimens in the British Museum of Natural History (BMNH) VF 16 and BMNH VF 18 to show different rates of shell expansion. **B,** paratype, BMNH VF 23, oblique apical view showing the internal septa.

Runnegar, 1976; Runnegar and Pojeta, 1985; Gubanov *et al.*, 1999).

Both of these morphological trends are repeated through the Early and Middle Cambrian (Peel, 1991b). Species referred to *Scenella* Billings, 1872 have widely expanded, limpet-like shells, although these are not universally accepted as molluscs (Yochelson and Gil-Cid, 1984; Landing and Narbonne, 1992). *Marocella* Geyer, 1986 includes several species formerly assigned to *Scenella* (*S. morenensis* Yochelson and Gil-Cid, 1984 and *S. antiqua* Kiaer, 1916), but here, too, there is uncertainty about affinity (Geyer, 1986; Evans, 1992). Morphologically similar shells of uncertain systematic position also occur in the Late Cambrian and Early Ordovician (Stinchcomb, 1986; Webers *et al.*, 1992; Webers and Yochelson, 1999). Lateral compression characterises the Early Cambrian *Stenotheca* Salter, 1859, some species of *Yochelcionella* Runnegar and Pojeta, 1974, and the Middle Cambrian genera *Mellopegma* Runnegar and Jell, 1976 and *Eurekapegma* MacKinnon, 1985. Peel (1991a, b) described a morphological series in the Early-Middle Cambrian *Eotebenna* Runnegar and Jell, 1976 in terms of increased penetration of soft bottom sediments. Culminating in the elongate *Eotebenna viviannae* Peel, 1991, in which convergence of the lateral areas produces separate anterior and posterior openings, this series can be directly compared with morphological adaptations in rostroconchs (Pojeta and Runnegar, 1976; Runnegar and Pojeta, 1985).

THE LAST HELCIONELLOIDS

Recently identified helcionelloids from the earliest Ordovician (Tremadoc) of Kazakhstan are the youngest undoubted members of the class. The specimens are typically helcionelloid in form, coiled through about one whorl. They are about 1-2 mm in length, laterally compressed so that width is about 30 % of length and with ornamentation of comarginal growth lines. The recent recognition of these Kazakhstan helcionelloids requires re-examination of the variety of Late Cambrian-Ordovician problematic isostrophic shells generally placed in the Bellerophontoidea (Gastropoda) or the cyrtonellid Tergomya (Runnegar and Jell, 1976; Peel, 1991b).

A recently described monoplacophoran mollusc from Silurian thermal vent deposits in the Ural Mountains of Russia (Little *et al.*, 1999) provides a challenge to current understanding of tergomyan or helcionelloid evolution.

Thermoconus shadlunae Little, Maslennikov, Morris, and Gubanov, 1999 (Fig. 5) is an unusually large (5-8 cm tall), straight-sided orthoconical shell with a sub-circular cross-section. A specimen with a broken apex reveals internal septa, which are concave towards the aperture (Fig. 5B). The external surface shows two distinct sculpture patterns, with rounded costae in the early stages and reticulate ornamentation with regularly spaced flanges in the late stage.

The shell form of *Thermoconus* is unlike other described tergomyans, but superficially similar tall cones are known in the Late Cambrian-Early Ordovician *Hypseloconus* Berkey, 1898 and *Knightoconus* Yochelson, Flower and Webers, 1973 (Webers *et al.*, 1992). Interpreted as tergomyan, *Thermoconus* represents the first evidence of the migration of this group from the shallow water environments in which it occurs from the Late Cambrian to the Devonian, to the deep-water environments that characterise most of its species at the present day.

While *Thermoconus* is quite reasonably interpreted as a tergomyan mollusc by Little *et al.* (1999), it lacks any muscle scars to substantiate the determination. In addition to their occurrence in the tergomyan *Knightoconus*, septa of the type found in *Thermoconus* are very common in some euomphaloidean gastropods (Gubanov *et al.*, 1995) and among other fossil and recent gastropod taxa (Yochelson *et al.*, 1973). Septation is also a characteristic feature of some helcionelloid molluscs such as *Helcionella chinensis* Walcott, 1905, *H.*? *insulcata* Rasetti, 1957 and *H. coreanica* Kobayashi, 1958. *Thermoconus* is unlike known Lower Palaeozoic gastropod limpets (*e. g.* Peel and Horny, 1999), but deep water or hydrothermal vent limpets are lacking in the geological record. The array of exotic gastropod limpets described from these habitats at the present day by McLean (1981, 1990) and later papers surely indicates that atypical gastropod limpets could have occurred in vent communities already in the Palaeozoic.

Thermoconus is much larger than any described helcionelloid, but this is a feature of Silurian molluscs in general when compared to their Cambrian progenitors (Runnegar and Pojeta, 1985), although some Tommotian helcionelloid molluscs have already attained a rather large size (see discussion above). Recently one of us (APG) collected possible helcionelloid molluscs, tentatively determined as *Helcionella* sp. with a size up to 8 cm, in the Upper Cambrian-Lower Ordovician Kruzhilikha Formation of the Severnaya Zemlya Archipelago (Arctic Siberia). If we interpret *Thermoconus* as a helcionelloid, it represents a relic of a group of molluscs that were abundant in the Early-Middle Cambrian but survived into the Silurian by taking refuge in the deep ocean. The scenario parallels the more recent history of the Tergomya.

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The Bellerophont Controversy Revisited

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Abstract: An old controversy reestablished itself in the late 1970s and early 1980s that focused on the systematic placement of the enigmatic Bellerophontoidea (informally, "bellerophonts"), a group of planispirally coiled, wholly fossil molluscs. The controversy embraced three fundamental concepts that are based on different philosophical interpretations of shell form, muscle scar patterns, and other preserved shell features: 1) all bellerophonts were monoplacophorans; 2) all bellerophonts were gastropods; and 3) some bellerophonts were monoplacophorans and some were gastropods. A review of the main issues appearing in the literature since the early 1980s indicates that these three philosophical divisions still exist and, indeed, have become entrenched. An examination of the relevant anatomical and shell features of recent gastropods and monoplacophorans, and comparison with preserved features in enigmatic fossil forms, convinces us that the bellerophontoideans and the coiled and high-domed "monoplacophorans" (Cyclomya) were gastropods. Only the flattened, spoon- and cap-shaped monoplacophorans (Tergomya) were true monoplacophorans. We present a hypothetical scheme for the morphological diversification of gastropods from early monoplacophorans that could account for Cyclomya, Bellerophontoidea, Patellogastropoda, and Prosobranchia.

Key Words: Gastropoda, Monoplacophora, Bellerophontoidea, functional morphology, phylogeny

One of the more polarizing arguments in malacology in the latter half of the 20th century has been termed the "bellerophont controversy". At the heart of this controversy is the question of whether the Bellerophontoidea, an extinct group of bilaterally symmetrical univalved molluscs, of which *Bellerophon* Montfort (Fig. 1) is a typical example, were gastropods or monoplacophorans. If they were monoplacophorans, they were untorted and exogastric (with the shell coiled over the head). If they were gastropods, they were torted and endogastric (with the shell coiled over the foot as in the majority of extant gastropods). The history of the controversy has been summarized many times and need not be repeated here. Readers interested in exploring the details might begin with summaries published by Yochelson (1967 – an excellent introduction to the natural history and evolution of thought on the bellerophonts), Harper and Rollins (1982), Peel (1985b), and Wahlman (1992).

TERMINOLOGY

We use the term "bellerophont" in an informal sense to designate both the Bellerophontoidea and the coiled Cyclomya; that is, any planispirally coiled, univalved mollusc that definitely is not a cephalopod. We also use the term "monoplacophoran" in an informal sense because several authors (Salvini-Plawen, 1980; Wingstrand, 1985; Peel, 1991; and Geyer, 1994) recently urged abandonment of that formal name. N. H. Ohdner (*In:* Wenz, 1940) introduced "Monoplacophora" with the intent that it would be an informal term separating the superfamily Tryblidioidea from the Polyplacophora. Knight (1952) formalized the name by including Monoplacophora as a gastropod order (Knight included the order Polyplacophora with the gastropods as well!). The fossil tryblidioids had long been considered patelliform gastropods with multiple muscle scars until Wenz hypothesized that they had been, in fact, untorted animals. The discovery of *Neopilina* Lemche (Lemche, 1957; also Lemche and Wingstrand, 1959) gave credence to that hypothesis.

Horný (1965a) established two new monoplacophoran subclasses, Tergomya and Cyclomya. Tergomya includes the cap-shaped or spoon-shaped monoplacophorans such as *Tryblidium* Lindström in which the plane of the muscle field lies outside the apical axis (a curved line marking the exact center of the shell during ontogeny) (Fig. 2A). Cyclomya includes a wide variety of coiled and uncoiled forms in which the plane of the muscle field intersects the apical axis (Fig. 2B). This appears to represent a distinct and natural morphological separation among shells exhibiting multiple pairs of muscle scars.

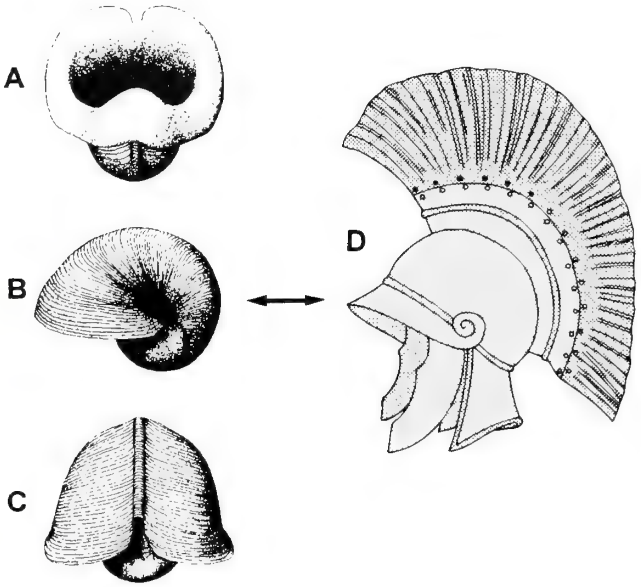

Fig. 1. *Bellerophon* Montfort, the isostrophically coiled mollusc that lent its name to the bellerophont controversy. **A** - Apertural view. **B** - Lateral view. **C** - Antero-dorsal view. Notice the similarity of the shell form to an ancient Greek helmet - **D**.

THE CONTROVERSY AND ITS EFFECTS

By the early 1980s many of those working with bellerophonts and/or molluscan phylogeny had encountered the puzzle of what to do with planispirally coiled, non-cephalopod, univalved molluscs bearing evidence of symmetrical, often multiple, muscles. As a result of quite different philosophies, these workers became polarized into three camps: group 1 – those who considered all bellerophonts to be monoplacophorans (Runnegar and Pojeta, 1974; Pojeta and Runnegar, 1976; Runnegar and Jell, 1976; Salvini-Plawen, 1980; Runnegar, 1981); group 2 – those who considered all bellerophonts to be gastropods (the majority of those who worked primarily on gastropod systematics and biostratigraphy); and group 3 – those who considered the bellerophonts a polyphyletic group that included both monoplacophorans and gastropods (Horný, 1963a, b, 1965a, b; Peel, 1972, 1974, 1976, 1980; Linsley, 1977, 1978a, b; Berg-Madsen and Peel, 1978). This third group considered that those bellerophonts having multiple sets of muscle scars were monoplacophorans whereas those with a single set of "columellar" scars were gastropods.

Harper and Rollins (1982) reviewed the controversy and critically examined the significance of shell structure, apertural re-entrants, parietal deposits, and muscle scars, all of which had been used at times by previous workers in distinguishing gastropods and monoplacophorans among the bellerophonts. All of these features have limitations in systematics, and at the time we felt muscle scars in particular were probably the least reliable single criterion on which to base a phylogeny. We concluded that placing bellerophonts and monoplacophorans in a single class based simply on shell form and muscle scar patterns was "tantamount to classifying bats, birds, and insects together because they all have bilateral symmetry and wings" (Harper and Rollins, 1982, p. 229).

Opinion in the paleontological community has changed little since the early 1980s. A review of the literature since mid-1982 indicates that group 1 still considers all bellerophonts to be monoplacophorans (Stanley, 1982; Runnegar, 1985; Runnegar and Pojeta, 1985; Signor, 1985; Geyer, 1994). Those in group 2 still consider the bellerophonts to be gastropods (McLean, 1984; Harper and Rollins, 1985; Kase and Nishida, 1986; Boucot *et al.*, 1986; Rohr and Yochelson, 1990; Frýda and Guitierrez-Marco, 1996; Ebbestad, 1999; and many others). Those in group 3 still consider the bellerophonts to be divided among the gastropods and monoplacophorans (Linsley and Peel, 1983; Peel, 1985a, b, 1993; Horný, 1986, 1993; Edlinger, 1988; Wahlman, 1992; Berg-Madsen and Peel, 1994).

The primary questionable contention that is driving the bellerophont controversy is that multiple pairs of symmetrical muscles in conjunction with the bilateral symmetry of the shell indicate a monoplacophoran affinity for all bellerophonts (Wenz, 1940; Runnegar and Jell, 1976; Salvini-Plawen, 1980; Geyer, 1994). This argument is based on the assumption that asymmetry is a necessary consequence of torsion, involving all internal and external organs, including the shell muscle. In effect, this argument ignores the innumerable biological investigations done over the past 200 years showing post-torsional symmetry of many gastropod species and their shell muscles, and even of organs such as the ctenidia, osphradia, and hypobranchial glands (as in many fissurelloideans). Yet the existence of multiple, symmetrical muscles has been used time and again as a valid systematic character allying such disparate molluscs as *Neopilina* Lemche and *Bellerophon* Montfort. For example, Rollins and Batten (1968), when confronted with conflicting morphological evidence, were persuaded to assign the sinus-bearing bellerophont *Sinuitopsis acutilira* (Hall) to the monoplacophorans based solely upon multiple symmetrical pairs of muscle scars.

TORSION AND ITS CONSEQUENCES

The argument that bellerophonts were untorted because asymmetry, as represented by helical coiling as well as asymmetry of soft parts, must be a necessary consequence of torsion has been reiterated by many authors (Ghiselin, 1966; Runnegar and Pojeta, 1974; Stanley, 1982;

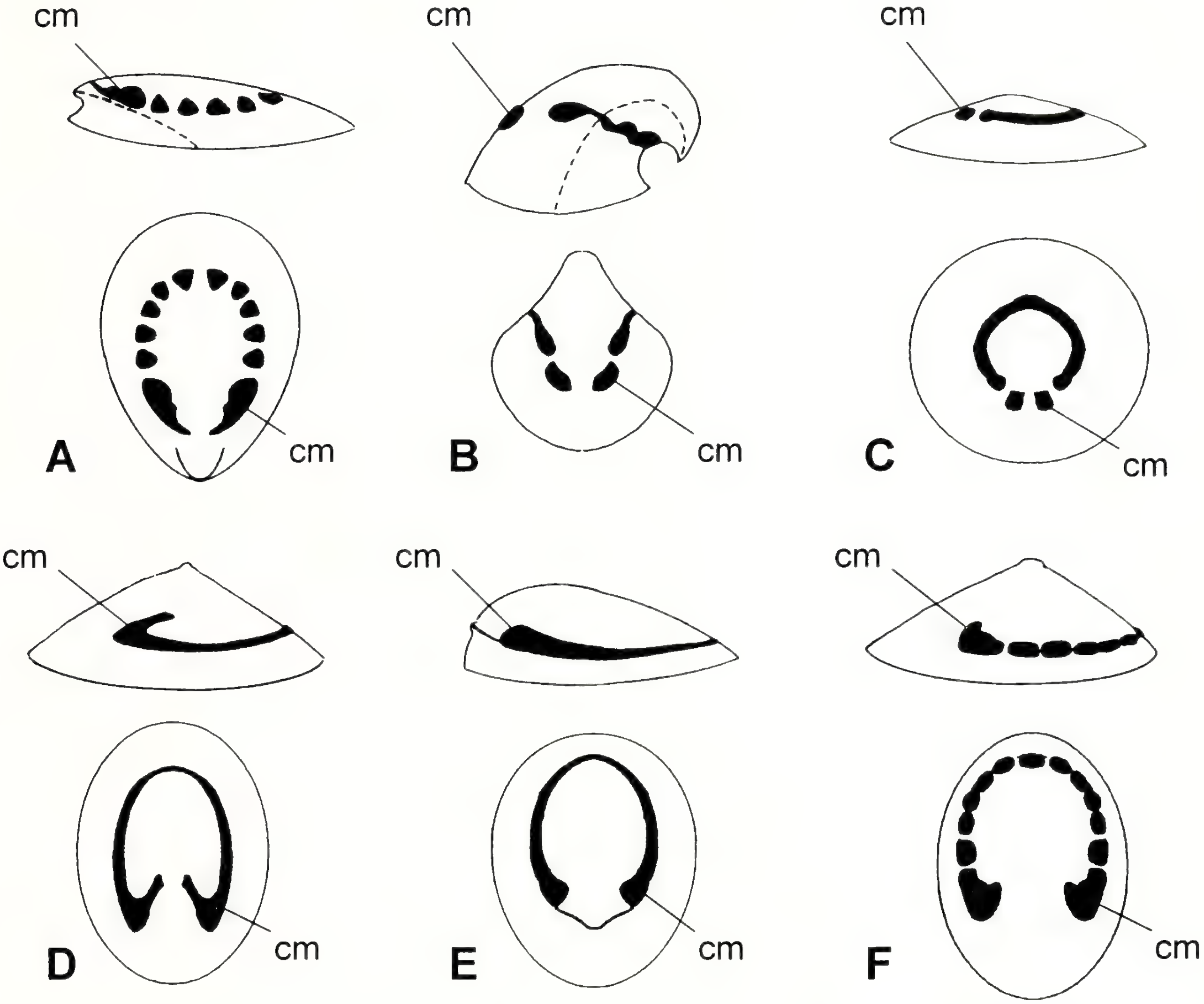

Fig. 2. Diagrams of representative tergomyan and cyclomyan monoplacophorans and patelliform gastropods, showing lateral and dorsal views of muscle scar patterns (black), including the discrete cephalic muscle (cm). **A** - Silurian tergomyan *Tryblidium* Lindström, showing the muscle field lying outside the apical axis (dashed line); **B** - Devonian cyclomyan *Cyrtonella* Hall, showing the muscle field intersecting the apical axis; **C** - Ordovician archinacelloidean *Floripatella* Yochelson; **D** - Recent fissurelloidean *Diodora* Gray; **E** - Recent acmaeoidean *Lottia* Gray; and **F** - Recent cocculinoidean *Cocculina* Dall.

Geyer, 1994, just to name a few). Linsley and Kier (1984), on the other hand, have argued that torsion and asymmetry are separate events in gastropod ontogeny; this has been shown to be the case by Bandel (1982). In fact, both the fossil record and the modern seas are full of prosobranch gastropods (torted) with symmetrical shells and opisthobranch gastropods and other molluscs (untorted) with helically coiled shells. Haszprunar (1988a, b) also found no primary correlation between torsion and helical coiling. He suggested that the occurrence of hyperstrophy in the protoconchs of higher gastropods argues for two independent processes, demonstrating that the direction of shell coiling is not correlated with the direction of torsion. We therefore reject the notion that bellerophonts must be untorted because they were isostrophically coiled.

APERTURAL RE-ENTRANTS

One of the most frequently used shell features for separating gastropods from monoplacophorans are slits, sinuses, and other re-entrants on the margins of the shell aperture. For example, the Pleurotomarioidea are best-known for having a deep, narrow slit near the middle of the whorl that is used to channel the exhalant current away from the inhalant currents. Linsley's fourth "law" of gastropod shell form states, simply, "Angulations or re-entrants on the aperture are usually indicative of inhalant or exhalant areas; inhalant areas will be directed as anteriorly as possible" (Linsley, 1977:200). This law makes sense only in motile animals – the gastropod has the advantage of sensing its environment in advance of its direction of motion. Because some bellerophonts have re-entrants situated laterally or postero-laterally, close to the shell coil, Starobogotov (1970), Berg-Madsen and Peel (1978), and others considered these forms monoplacophorans. However, Linsley (1977) admitted that he did not consider all gastropods when formulating this "law." Harper and Rollins (1982) argued that the fourth "law" was not necessarily applicable to limpets and other bottom clampers. Many limpet gastropods have "re-entrants" all around the shell, related to coarse radial ribs that end at the shell margin in small concave, v-shaped emarginations. Also, the inhalant and exhalant currents in limpets are not necessarily positioned anterior and posterior as they are in the helically coiled gastropods. In *Patina*, for example, the inhalant current enters the pallial groove along the length of the gill skirt and exits anteriorly through the right side of the nuchal cavity (Fretter and Graham, 1962). *Patella* similarly draws water in along the whole margin of the mantle, but expels it ventrally. In the caenogastropod limpet *Crepidula*, the current enters the mantle cavity on the left and leaves on the right – both inhalant and exhalant currents are lateral.

We agree that apertural reentrants should be a good indication of whether a mollusc is a monoplacophoran or a gastropod, but only if the animal is truly motile. However, of all the coiled "monoplacophorans" in which the muscle scars are known, none is what we would call a truly motile animal. We envision most of the cyclomyans as limpets or shell clampers (as opposed to those that retract the body into the shell like most prosobranchs). These forms commonly have large, tangential apertures and loosely coiled shells, as opposed to the tight coils and radial apertures of forms such as *Bellerophon*. They are also often covered with epibionts. This suggests to us that these forms were functionally limpet-like (although some very motile gastropods harbor shell-covering epibionts). The most functionally advantageous place for inhalant currents would be close to the gills and relatively far from the exhalant currents. As in *Patina, Patella*, and *Crepidula*, this could easily have been just about anywhere on the shell. We conclude that presence of lateral re-entrants is not a definitive criterion for separating limpet-like monoplacophorans and gastropods.

It should be noted that certain cyclomyans have small re-entrants on the aperture beneath the shell coil (*e. g. Neocyrtolites* Horný – see Horný, 1993, Pl. 1, Figs. 7, 8). If these functioned as inhalant currents, the animal almost certainly was exogastric. However, these features commonly are very small and probably would not have been very effective for channeling currents into a large aperture. Their function is uncertain, but may have been associated with an operculum or some other feature.

COMPARISONS OF MUSCLE SCARS

The suggestion that multiple muscles are a hallmark only of the monoplacophorans has no basis in fact. Most non-helically coiled limpet gastropods (fissurelloideans, patellogastropods, cocculinoideans, etc.) have horseshoe-shaped muscle fields with a distinctive break at the anterior end that defines the position of the mantle cavity. In the Patellogastropoda, the muscle field appears to be continuous, but in fact it is arranged as a series of discrete muscle bundles separated by narrow clefts. In the Cocculinoidea the muscle bundles display a widely varying degree of separation (Haszprunar, 1987, 1988a). Thiem (1917) showed that the clefts allow space for afferent vessels to transfer blood from the foot and visceral mass into spaces in the mantle skirt where oxygenation takes place. The degree of separation appears to be directly related to the efficiency of the gills (Fretter and Graham, 1962). The Fissurelloidea have two very efficient ctenidia and no pallial gills, so the muscle bundles have little division (Fig. 2D). The Patellidae and Lottiidae exhibit well-developed pallial gills, and the Lottiidae have one ctenidium, so that oxygenation occurs easily. There are few afferent vessels and few muscle divisions (Fig. 2E). The Acmaeidae and Lepetidae have one ctenidium that is not very effective, and no pallial gills, so that pallial respiration via afferent vessels is necessary for adequate blood oxygenation. Species of these families commonly exhibit numerous muscle clefts. The separation of the individual muscle bundles is especially noticeable in the Cocculinoidea (Haszprunar, 1987, 1988a) (Fig. 2F).

Based on innervation of shell muscles in limpet gastropods, Haszprunar (1985) showed that there is only a single pair of muscles. However, he also indicated the possibility that a single pair of shell muscles divided into discrete bundles might be the primitive condition shared by monoplacophorans (Haszprunar, 1988b, p. 374).

It seems likely that multiplicity of muscles (regardless of division) is a normal condition in animals that pull the shell down to the substrate, as opposed to those that retract into their shells. It is also likely, based on those animals with discrete muscle bundles and those with seemingly solid horseshoe-shaped muscle masses, that environmental conditions and habitat diversity played a large part in the origination of the particular muscular condition. More muscle mass (the "solid" horseshoe) probably was an adaptation either to ward off predation or to the vagaries of near-shore conditions (storms, rough seas, desiccation episodes, etc.). In either case, it would allow the animal to clamp the shell more tightly around the body and foot. If this is the case, then discrete, multiple muscles must indicate a lack of need, or less need, for such muscle mass. Indeed, modern monoplacophorans, which live in deep, quiet water, tend to have weakly developed muscles that leave no scars on their shell interiors. The Cocculinidae, which probably have the best set of discrete muscles among Recent limpet gastropods, also occur in deep water (McLean, 1987; Haszprunar, 1987). On the other hand, the fossil tryblidiids, which lived in shallow water and probably functioned in much the same fashion as modern limpet gastropods, had strong muscles inserted well into the shell.

The largest of the paired muscle bundles in modern limpet gastropods appears at the anterior end of the muscle field and is associated with retraction of the head. In at least two Recent limpet groups, Fissurelloidea and Lepetelloidea, this muscle pair has hook-like protrusions on the interior side of the bundle. McLean (1984) suggested these hook-like protrusions in the fissurelloideans mark the position of muscles that control the ctenidia. Although this is possible, we feel it is unlikely because similar hook-like protrusions occur on the cephalic muscles of many other limpets that have only one, or no, ctenidium. The Lepetelloidea, for example, have several pallial leaflets of secondary origin restricted to the right post-torsional side of the mantle skirt, outside the shell muscle field (Haszprunar, 1988b). The hook-shaped muscles also occur in Polyplacophora and in many Paleozoic monoplacophorans such as *Tryblidium* (cm in Fig. 2A). For McLean's hypothesis to be correct, the ctenidia in *Tryblidium* and other monoplacophorans would have to be situated in an anterior mantle cavity which, of course, does not exist in untorted molluscs. Alternatives include: 1) the hook-shaped muscles in fissurelloideans had distinctly different functions in monoplacophorans and, following torsion, the gastropod limpets adapted the muscle for use with the ctenidia; 2) the hook-shaped muscles are convergent features in the two lineages with no homologous functions; and 3) the hook-shaped muscles are homologous features but have a different function than suggested by McLean (1984). We prefer the third option, but are uncertain of their function. The hook-shaped muscles are likely related to retraction of the head and, possibly, anchoring of the buccal muscles. Purchon (1977, p. 467), in discussing the muscles of Recent monoplacophorans, described a "complex series of muscles inserted into the shell anteriorly on either side of the mouth [that] serve to move the lips of the mouth, the velar lobes, the post-oral tentacles, and the radular apparatus." Although the cephalic muscle area of *Neopilina* Lemche does not look much like that of *Tryblidium* Lindström, in all likelihood they are homologous, as well as analogous, structures. It may be that the change from a shallow water, presumably herbivorous mode of life in Paleozoic monoplacophorans to a deep water, deposit feeding mode of life in Recent forms accounts for the differences.

We should, therefore, be able to use the relatively larger cephalic muscles in cap-shaped or spoon-shaped monoplacophoran and gastropod limpets to recognize the anterior ends of the shells. In *Tryblidium* Lemche (Fig. 2A) the cephalic muscles occur near the shell apex, indicating the animal's exogastric nature. In the gastropod limpets (Figs. 2C-F) the cephalic muscles should occur on the end opposite the shell apex (endogastric). The same is true of the archinacelloideans (Fig. 2C) which, despite their apparent tergomyan appearance, generally are considered to be gastropods (Starobogotov, 1970; Harper and Rollins, 1982; Yochelson, 1988; Mazaev, 1998). (Note, however, that in many limpet gastropods, the "coil" is anterior or centralized. Therefore, the terms "endogastric" and "exogastric" are essentially meaningless without reference to soft anatomy.)

We suggest these cephalic muscles also occur in the cyclomyans as the pair of subcircular to oblong muscle scars on the shell dorsum, farthest from the shell coil (Figs. 2B, 3A). Dzik (1981) reconstructed the cyclomyan *Sinuitopsis* Perner with these muscles acting as retractors for an operculum on the trailing foot of the animal. We find this highly unlikely, not because there is no evidence for an operculum in the cyclomyans, but simply because the muscle pads appear to be homologous with the cephalic muscles in tergomyans, archinacelloideans, and modern limpet gastropods. If the cyclomyans were exogastric, like the tergomyans, the cephalic muscles of these animals then would have been attached to the back of the shell above the visceral hump and well away from the head, which makes no sense. The head must have been situated below the cephalic muscles and on the opposite end of the body whorl from the coil, indicating the cyclomyans were endogastric. While this is not an impossible situation in monoplacophorans, in view of most efficient muscle function a more likely explanation is that the cyclomyans were gastropods.

We envision a complete range of shell muscles arranged in a functional sequence (Fig. 4) based on coiling parameters and apertural characters, which in turn were very probably based on mode of life and physical environment. According to figure 4, the uncoiled cyclomyans like *Cyrtonella* Hall were the most limpet-like forms, grading (functionally, but not necessarily phylogenetically) through the cyrtolitiform (*e. g. Cyrtolites* Conrad) and sinuitiform (*e. g. Sinuitopsis* Perner which has an anal emargination like the pleurotomarioideans) cyclomyans to the bellerophontoideans (*e. g. Bellerophon* Montfort). Forms such as *Sinuites* Koken, *Syvestrosphaera* Peel, and *Carcassonella* Horný and Peel were intermediaries, retaining the cephalic muscles of the limpets while accomplishing retraction through application of lateral retractor muscles similar to the helically-coiled gastropods. These forms may have had a completely different mode of life from both the limpets and bellerophontoideans. In fact, Horný (1996) has described *Sinuites* Koken as a semi-infaunal gastropod based on secondary shell layers similar to those in the bellerophontoidean *Euphemites* Warthin (see Harper and Rollins, 1985). Pronounced variation in shell muscle position and pattern correlated with degree of shell coiling is also recognized in the Paleozoic platyceratids (Rollins and Brezinski, 1988).

PARIETAL DEPOSITS

Harper and Rollins (1982) and Wahlman (1992) were convinced that the most reliable criterion for assignment of any isostrophically-coiled mollusc to the Gastropoda was the presence of a massively developed parietal deposit. Such deposits are most easily viewed as functionally enhancing shell stability by placing the bulk of shell weight over the posterior foot, and less reasonable if elevated over the head of the animal. Rollins (1966) noted that the parietal pad of the bellerophont *Ptomatis* also displayed a central cleft that most likely rested directly upon the foot. Massive parietal deposits, however, are variously developed in bellerophonts. When present, they appear to confirm torsion and justify ready assignment to the Gastropoda. Their absence, or even the presence of a thin inductural wash, is inconclusive. As noted by Pojeta and Runnegar (1976), thin secondary shell deposits might have been secreted by epithelial tissue near the head of the animal.

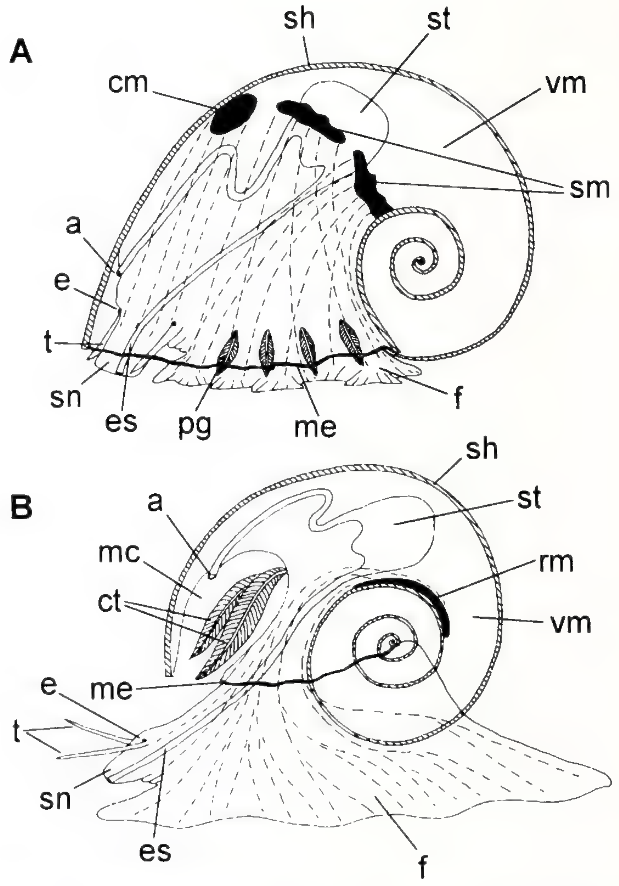

Fig. 3. Proposed configurations of head, foot, visceral mass, and various organs for: **A** - *Cyrtolites*, a representative cyclomyan; and **B** - *Bellerophon*, a representative bellerophontoidean. a - anus; cm - cephalic muscle; ct - ctenidia; e - eye; es - esophagus; f - foot; mc - mantle cavity; me - mantle edge; pg - pallial gill; rm - retractor muscle; sh - shell; sm - shell muscles; sn - snout; st - stomach; t - tentacle; vm - visceral mass.

DISCUSSION

It has become obvious that the monoplacophorans, at least those represented by *Neopilina* Lemche, do not represent the molluscan archetype (hypothetical ancestral mollusc). All of those forms we know or suspect to be monoplacophorans are too highly specialized, with their serial pairs of organs and muscles, the mantle covering the entire animal, and a pallial groove that completely encircles the body substituting for a centralized mantle cavity. However, given the close anatomical relationship of monoplacophorans and polyplacophorans, this must be the plesiomorphic condition from which the gastropods arose. Haszprunar (1988b) suggested that a shallow mantle cavity, with a concomitant degree of respiration in the mantle roof and margin, is the primitive condition in gastropods. In addition, Haszprunar believes the subpallial cavity was probably used for respiration until the gastropods evolved secondary gills. The fossil record suggests otherwise, as shown below.

If, in fact, the fossil record is good enough, we should be able to see many of the morphological changes that evolved throughout the Phanerozoic in the tergomyans, cyclomyans, and bellerophontoideans. And indeed, we can. In figure 4 we show a series of hypothetical monoplacophoran and gastropod forms that are simply generalized models based on actual fossils such as *Tryblidium* Lindström, *Cyrtolites* Conrad, and *Bellerophon* Montfort. We envision a series of anatomical changes that occurred relatively rapidly in geologic time. Following torsion (Fig. 4, fundamental change A) the archetypal gastropod was a torted monoplacophoran having serially arranged muscles and organs, including multiple gills in a pallial groove surrounding the body. At some later time the population of archetypal gastropods gave rise to forms that increased the

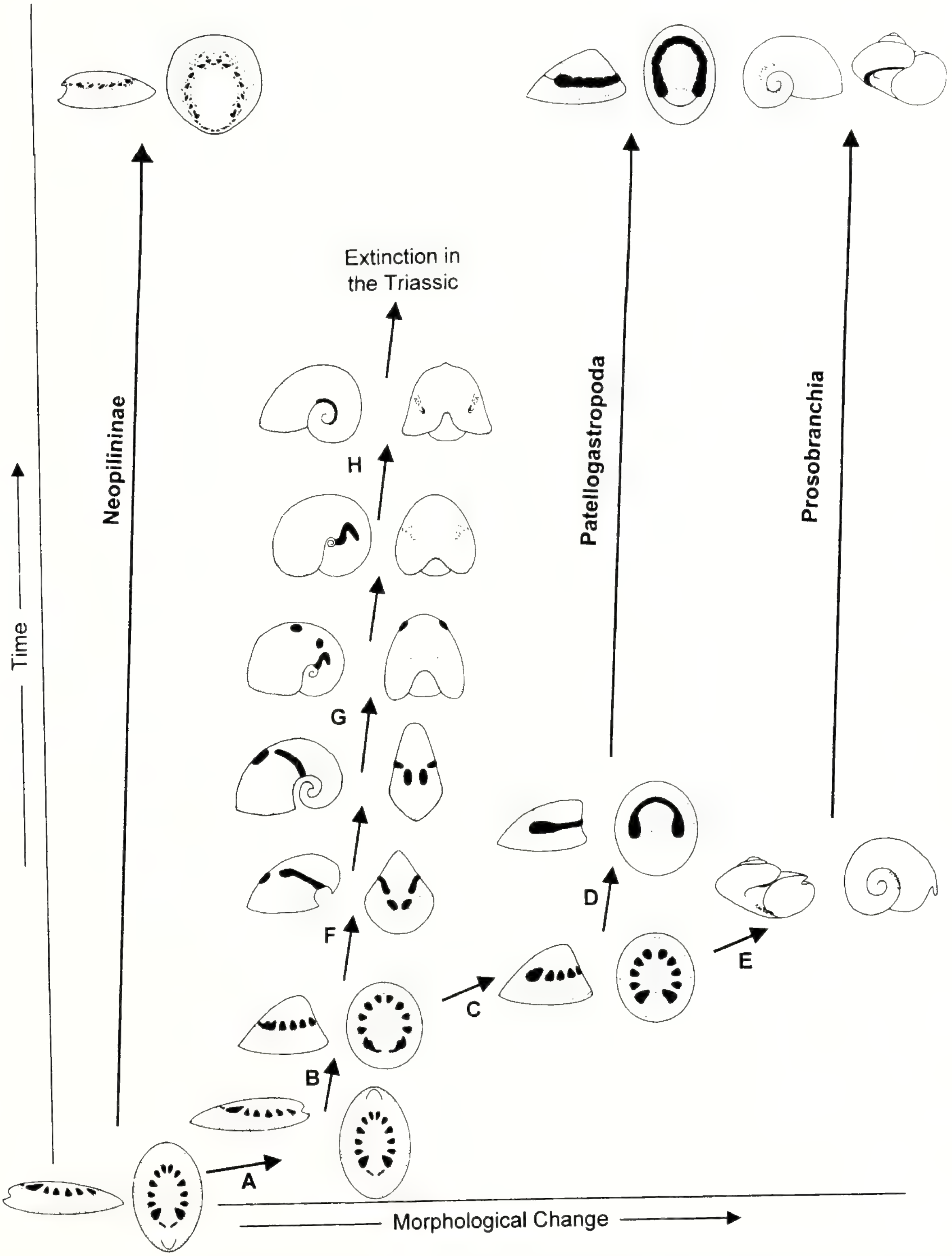
Extinction in the Triassic
Time
Neopilininae
Patellogastropoda
Prosobranchia
A
B
C
D
E
F
G
H
Morphological Change

Fig. 4. Diagram illustrating a hypothetical generalized evolutionary scheme for the gastropods and monoplacophorans. Muscle scars are shown in black, stippled where hidden behind shell. Dotted lines indicate proposed extent of the mantle/pallial cavity. Letters designate fundamental changes in anatomy. **A** - torsion; **B** - vertical expansion of the shell; **C** - development of an anterior mantle cavity; **D** - decrease in afferent vessels, resulting in fusion of the muscles to a horseshoe; **E** - helical coiling; **F** - isostrophic coiling; **G** - development of an anterior mantle cavity; and **H** - reduction of shell muscles to two retractors.

height of the shell (B), allowing for enlargement of the pallial groove. Certain of these high-domed gastropods experimented with centralizing the mantle cavity and reducing the number of gills (C). This allowed a decrease in the number of afferent vessels and a consolidation of the discrete shell muscles into a horseshoe (D). These gastropods eventually gave rise to the Patellogastropoda, which are distinct from all other archaeogastropods (see Lindberg, 1988 for a review of this gastropod order). Whether or not there was a coiled intermediate in this lineage, as some authors have suggested, is beyond the scope of this discussion.

High doming and centralization of the mantle cavity also allowed a separate lineage to experiment with coiling, particularly helical coiling. These forms gave rise to the most successful group of molluscs, the prosobranchs.

Meanwhile, the original group of high-domed archetypal gastropods also were "experimenting" with coiling, probably as a means to increase the amount of visceral mass without making the shell unstable and/or to afford greater protection of soft organs within the shell, perhaps concomitant with a more motile existence. These gastropods, which we have been calling cyclomyans, retained the plesiomorphic pallial groove and multiple gills of the ancestral gastropods. Through time these molluscs enlarged the pallial groove and concomitantly moved the shell muscles farther back into the shell. This provided a morphological setting that would lead, eventually, to the independent development of the centralized mantle cavity. Once this important feature was attained, the cyclomyans went through an adaptive radiation that resulted in an explosion of forms within the morphospace of the isostrophically-coiled shell. This radiation, linked to probable predation pressures and adaptive changes to the physical environment, forced modification in the shell from evolute, open-coiled, limpets to tightly involute, compact animals that spread out into a variety of adaptive niches - the Bellerophontoidea. Unfortunately, as successful as they were, the bellerophont lineage decreased substantially in diversity toward the end of the Paleozoic and ultimately became extinct, but well into the Triassic, not at the biotic crisis of the Permian extinction.

SUMMARY AND CONCLUSIONS

During the 1970s and early 1980s, a controversy concerning the systematic placement of the Bellerophontoidea ("bellerophonts") – a group of planispirally coiled marine molluscs known only from the fossil record and commonly considered to be primitive gastropods - emerged in the paleontologic literature. Although some points of contention had been raised as early as the 1940s, the primary concern now focused upon interpretation of multiple, symmetrical muscle scars preserved on internal molds (steinkerns) of certain Bellerophontoidea. One camp insisted that, because multiple, symmetrical muscles suggest metamery, the Bellerophontoidea must have been untorted and, therefore, were monoplacophorans rather than gastropods. Another group insisted that "bellerophonts" were gastropods. Yet a third camp argued that single pairs of muscles and various gastropod-like features of some "bellerophonts" indicate that many of these animals were gastropods. They conceded that other Bellerophontoidea were monoplacophorans and, although distinguishing between the separate lineages would be difficult, it could be done by analyzing all the shell features and not just the muscle scars (which were notoriously rare). In 1982 we presented evidence that the various arguments for "bellerophonts-as-monoplacophorans" were specious and concluded that all "bellerophonts" were gastropods. A reexamination of that evidence and consideration of research on these molluscs since 1982 has failed to convince us otherwise.

Following torsion, the archetypal gastropod was a torted monoplacophoran having serially arranged muscles and organs, including multiple gills in a pallial groove surrounding the body. At some later time the population of archetypal gastropods gave rise to forms that increased the height of the shell, allowing for enlargement of the pallial groove and centralization of the mantle cavity, and eventual development of the Patellogastropoda. High doming and centralization of the mantle cavity also allowed a separate lineage to experiment with helical coiling leading to the very successful Prosobranchia. The original group of high-domed archetypal gastropods also "experimented" with coiling, and this group, which we have been calling cyclomyans, retained the plesiomorphic pallial groove and multiple gills of the ancestral gastropods. This provided a morphological setting that would lead, eventually, to the independent development of the centralized mantle cavity and the adaptive radiation of the Bellerophontoidea.

The evolutionary scenario we described is speculative, of course, but it does have its basis in the fossil record and in comparative anatomy. We conclude, as we did previously (Harper and Rollins, 1982), that the Cyclomya and Bellerophontoidea were gastropods rather than monoplacophorans, and Cyclomya should be retained as a suborder of Bellerophontina. Only the Tergomya were, and are, true monoplacophorans.

It should be apparent that there is no reason to abandon the term Monoplacophora. Even though it originated as an informal name, and recently became a "garbage can" term for an unlikely grouping of exotic molluscs and, perhaps, non-molluscs, Monoplacophora should be retained as a formal class name. The taxonomic scope of this term has come full circle - originally proposed to include only the Tryblidiidae, it once again can be defined as the Tryblidiidae and associated Recent neopilinids. The term Tergomya then is synonymous with Monoplacophora and should be abandoned. Cyclomya should be retained as a suborder of Bellerophontina.

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Cambrian Pelecypoda (Mollusca)

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Abstract: The record of Cambrian bivalved organisms that have been placed in the Mollusca includes undoubted pelecypods and other bivalved shells. This record is reviewed herein. Additional topotype material of Delgado's (1904, Communicacoes da Commissao do Servico Geologico de Portugal 5:307-374) Lower Cambrian specimens from Portugal shows that they are distorted brachiopods. Restudy of Zhang's (1980a, Bulletin Chinese Academy of Geological Sciences, Series 8, 1:1-19; 1980b, 26th International Geological Congress 4:121-129) type specimens from the Lower Cambrian of China shows that most of them are also brachiopods. New specimens of *Tuarangia* MacKinnon indicate that this genus is a Middle-Upper Cambrian pelecypod.

Key Words: Cambrian, Pelecypoda, Brachiopoda, *Fordilla, Pojetaia*, Mollusca, bivalves

Over the past generation, a great deal of new information and data have been added to our knowledge of Cambrian and Ordovician pelecypods; much of this new information is summarized by Babin in this volume. My paper concentrates on the expanding knowledge of Cambrian pelecypods.

Undoubted Cambrian pelecypods are now known from North America, Europe, Greenland, Asia, Africa, and Australia. However, the stratigraphic range of the class in the Cambrian, which bivalved taxa are pelecypods and which ones are not, and how the Cambrian pelecypods are related to Ordovician taxa, are all questions under study by various workers who have reached differing conclusions.

Much recent work in Europe and South America has shown that the great Ordovician radiation of pelecypods began in Arenigian time about 10 million years sooner than previously thought. Thus, it is reasonable to assume that Cambrian rocks hold part of the key to understanding Ordovician pelecypods.

The Ordovician radiation causes us to debate the number of subclasses of pelecypods that should be recognized. However, by the end of Ordovician time almost all modes of life exploited by pelecypods were present — deposit feeding, filter feeding, epifaunal byssal attachment, burrowing, semi-infaunal byssal attachment, nestling, boring and, probably, swimming; ligament types and dentition were also highly varied.

EARLY CAMBRIAN TAXA

The known Cambrian stratigraphic range of pelecypods is being filled in with records now ranging from the Early Cambrian through the early Late Cambrian.

In a previous summary of information about Cambrian pelecypods to that date (Pojeta, 1975) I came to the conclusion that the only undoubted Cambrian pelecypod was *Fordilla troyensis* Barrande, 1881 (Fig. 1A,C,D). I noted that *Lamellodonta simplex* Vogel, 1962, probably was an obolellid brachiopod, a decision later confirmed by Havlicek and Kriz (1978). In the 1975 paper, I gave the age of *Fordilla* as Early Cambrian. More recently, Geyer and Streng (1998:87) indicated the age of *Fordilla* as "early late Early Cambrian to the late Early Cambrian (probably equivalents of the Siberian Atdabanian and Botoman[ian]..." Runnegar and Pojeta (1992:117) indicated *Fordilla* was known from rocks as old as Tommotian in Siberia (Fig. 2).

My 1975 summary noted that *Fordilla* was known from Lower Cambrian rocks in New York State, Newfoundland, Denmark, and Greenland. The genus has subsequently been found in Lower Cambrian rocks of the Siberian platform (Krasilova, 1977, 1987; Jermak, 1986) and eastern Germany (Elicki, 1994); Geyer and Streng, (1998:88) suggested that the German *Fordilla* could be better assigned to *Pojetaia*. In addition, undescribed material is known from Labrador.

The interpretation of *Fordilla* as a pelecypod was based on articulated material and internal molds that show pelecypod muscle scars. Subsequently, teeth and ligament groove fillings have been found (Pojeta, 1978). Subsequent workers accepted *Fordilla* as a pelecypod, documented the constancy of the muscle scar patterns, provided data about

Fordilla's shell microstructure, and added information about its geographic distribution in Early Cambrian time (Runnegar and Pojeta, 1992; Hinz-Schallreuter, 1995; Cope, 1996; Geyer and Streng, 1998; Cope and Babin, 1999).

Yochelson (1978) treated *Fordilla* as having originated from a hypothetical unknown ancestral mollusk separately from the origin of the Pelecypoda from the same ancestor. Yochelson (1981) preferred to treat *Fordilla* as molluscan *incertae sedis*, until more information about the genus became known. The authors cited herein have provided that new information.

The other undoubted Early Cambrian pelecypod is *Pojetaia runnegari* Jell, 1980 (Fig. 1E-H), first described

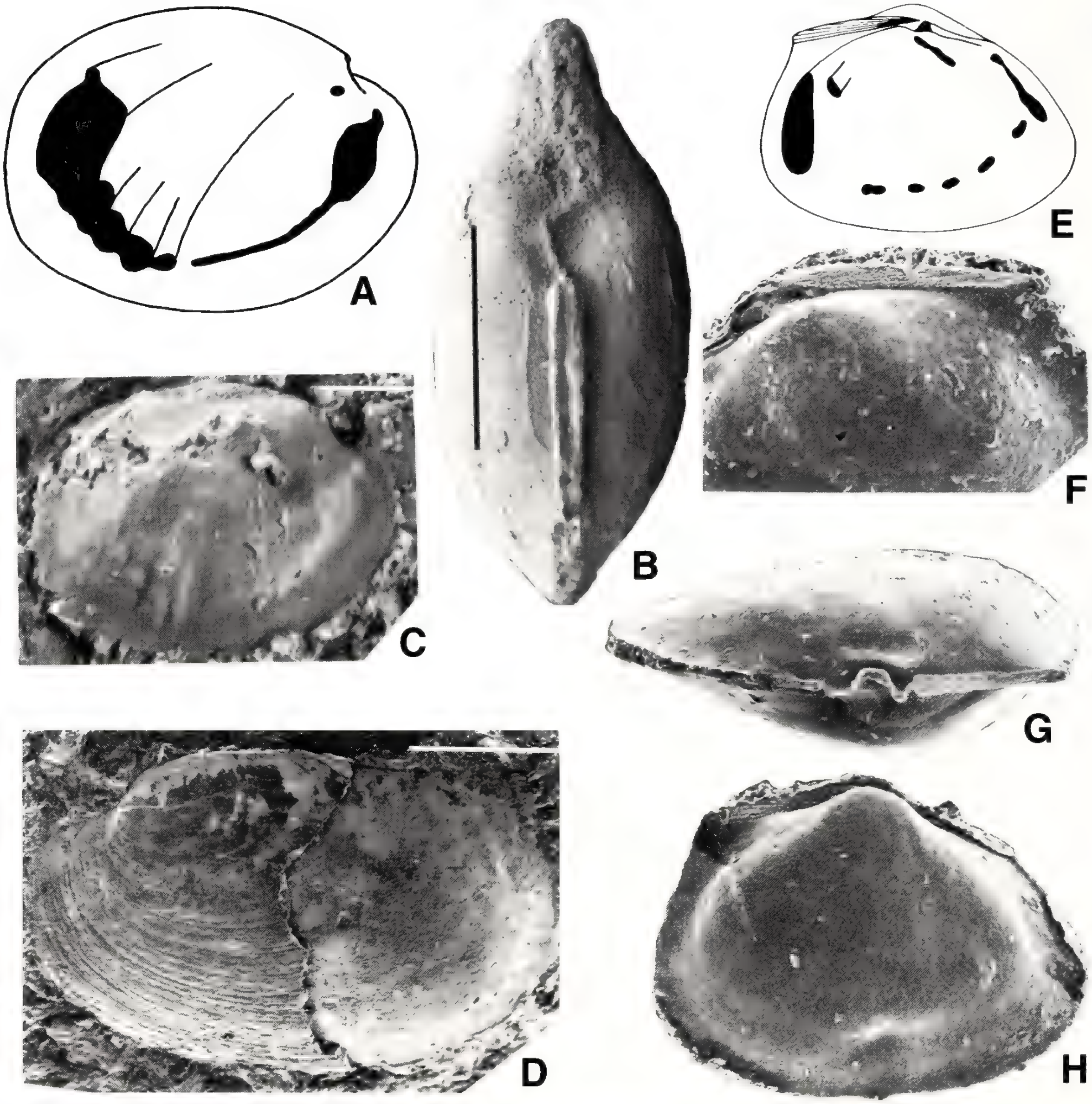

Fig. 1. Lower Cambrian pelecypods *Fordilla troyensis* Barrande (A, C, D), *F. siberica* Krasilova (B), and *Pojetaia runnegari* Jell (E-H). All scale bars equal one millimeter. **A,** right valve composite drawing of the muscle scars as seen on internal molds. Drawing by B. Runnegar. **B,** dorsal view of internal mold showing trace of one tooth in each valve and the filling of the ligament space, Lower Cambrian, Siberia. **C,** right valve internal mold showing most of the muscle scars, Lower Cambrian rocks, New York. **D,** left valve of partially shelled specimen showing growth lines, Lower Cambrian, Greenland. **E,** composite reconstruction of the muscle scars, modified from Runnegar and Bentley (1983); published with permission of *The Journal of Paleontology*. **F,** dorsal part of left valve showing ligament area and teeth, specimen 1 mm long, Lower Cambrian, Australia. **G,** dorsal view of internal mold showing trace of one tooth in each valve, anterior to left, specimen 1.2 mm long, Lower Cambrian, Australia. **H,** right lateral view of internal mold. The apparent texture on the surface was interpreted (Runnegar and Bentley, 1983) as impressions of crystals on the inner surface of the shell, specimen 0.9 mm long, Lower Cambrian, Australia.

from the Lower Cambrian rocks of Australia. This species was intensively studied by Runnegar and Bentley (1983), who dealt with all aspects of its morphology from prodissoconch I to shell microstructure. Runnegar and Pojeta (1992) added some information about comparative shell microstructure, stratigraphic distribution, and probable synonyms of both *Pojetaia* and *Fordilla*. *Pojetaia* has been widely accepted as the second well-documented Early Cambrian pelecypod. The genus is now known from Lower Cambrian rocks in South Australia; New South Wales, Australia; Anhui Province, China; Henan Province, China; Bornholm, Denmark; Morocco (Geyer and Streng, 1998); and, questionably from Newfoundland (Landing and Westrop, 1998). Geyer and Streng (1998:89) provided a table comparing the six named species of *Pojetaia*. Runnegar and Pojeta (1992:117) noted that *Fordilla* and *Pojetaia* are about coeval in their known first occurrences.

The relationships of *Pojetaia* and *Fordilla* to each other and to Ordovician pelecypods have been under frequent discussion. Making comparisons to Ordovician pelecypods, Pojeta and Runnegar (1985) regarded *Pojetaia* as a palaeotaxodont and *Fordilla* as an isofilibranch. Runnegar and Pojeta (1992) placed the two genera in a monophyletic grouping, Fordillidae Pojeta, 1975, based on their similar shell microstructure. Hinz-Schallreuter (1995) followed Runnegar and Pojeta (1992) and placed *Pojetaia* in the Fordillidae. Geyer and Streng (1998) and Cope (1996) treated *Pojetaia* as a palaeotaxodont. Cope (1996) noted that most authorities regard *Fordilla* as a pelecypod, but he doubted its assignment to the Isofilibranchia, as did Waller (1998). Cope and Babin (1999:175) noted: "Prolonged debate on the affinities of the genus *Fordilla*...has finally been settled and it is now regarded as one of the two known early Cambrian bivalve genera."

Runnegar and Pojeta (1992) and Geyer and Streng (1998) discussed the various Lower Cambrian taxa that have been assigned to the Pelecypoda. They noted probable synonyms and taxa that are probably not pelecypods. Herein, new information is provided on two occurrences, in Portugal and China, indicating that certain Lower Cambrian bivalves once regarded as pelecypods should be assigned elsewhere.

From the Lower Cambrian rocks of Portugal, Delgado (1904) described nine species, placed in six genera, of what he regarded as pelecypods. For years since his publication, Delgado's material was little studied or noticed. Teixeira (1952) restudied Delgado's material, placed three of the species defined by Delgado in "Lamellibranchia?," and the remainder in the single species *"Modiolopsis" bocagei* Delgado, which Teixeira regarded as a pelecypod.

In 1904, Delgado sent C. D. Walcott identified topotype specimens of *Modiolopsis bocagei* and *Davidia dollfusi* Delgado; these are figured herein for the first time (Fig. 3G, H). These specimens are probably deformed brachiopods; they clearly show cardinalia in the presumed brachial valves at the midlength of the hinge margin indicating a possible articulate brachiopod (identified by J. T. Dutro, Jr., who suggested that the cardinalia could be called socket ridges in these specimens). Thus, it seems likely that all of Delgado's "species" of Early Cambrian pelecypods are deformed brachiopods; the other elements of the fauna described by Delgado are also clearly deformed.

Zhang (1980a, b) described four new genera, seven new species, and two new families of bivalved creatures; these were first proposed as *nomina nuda* (Zhang, 1979). His material is from the Tianheban Formation, a *Redlichia* trilobite-bearing Lower Cambrian unit at Zhongbao Village, Xianfeng County, Hubei Province, China; Zhang treated the bivalves from Hubei as pelecypods. Zhang (pers. comm., June 1996) noted that the Hubei fauna may correlate with

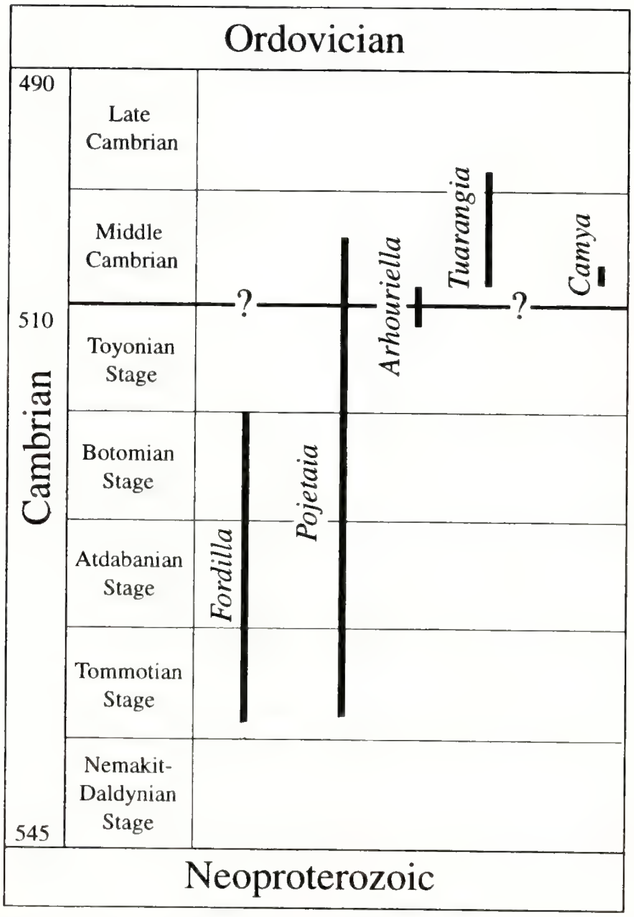

Fig. 2. Stratigraphic distribution of Cambrian pelecypods. The numbers in the left hand column indicate millions of years ago. The Early-Middle Cambrian boundary is queried because there is debate about where to place it (Landing *et al.*, 1998; Geyer, 1998).

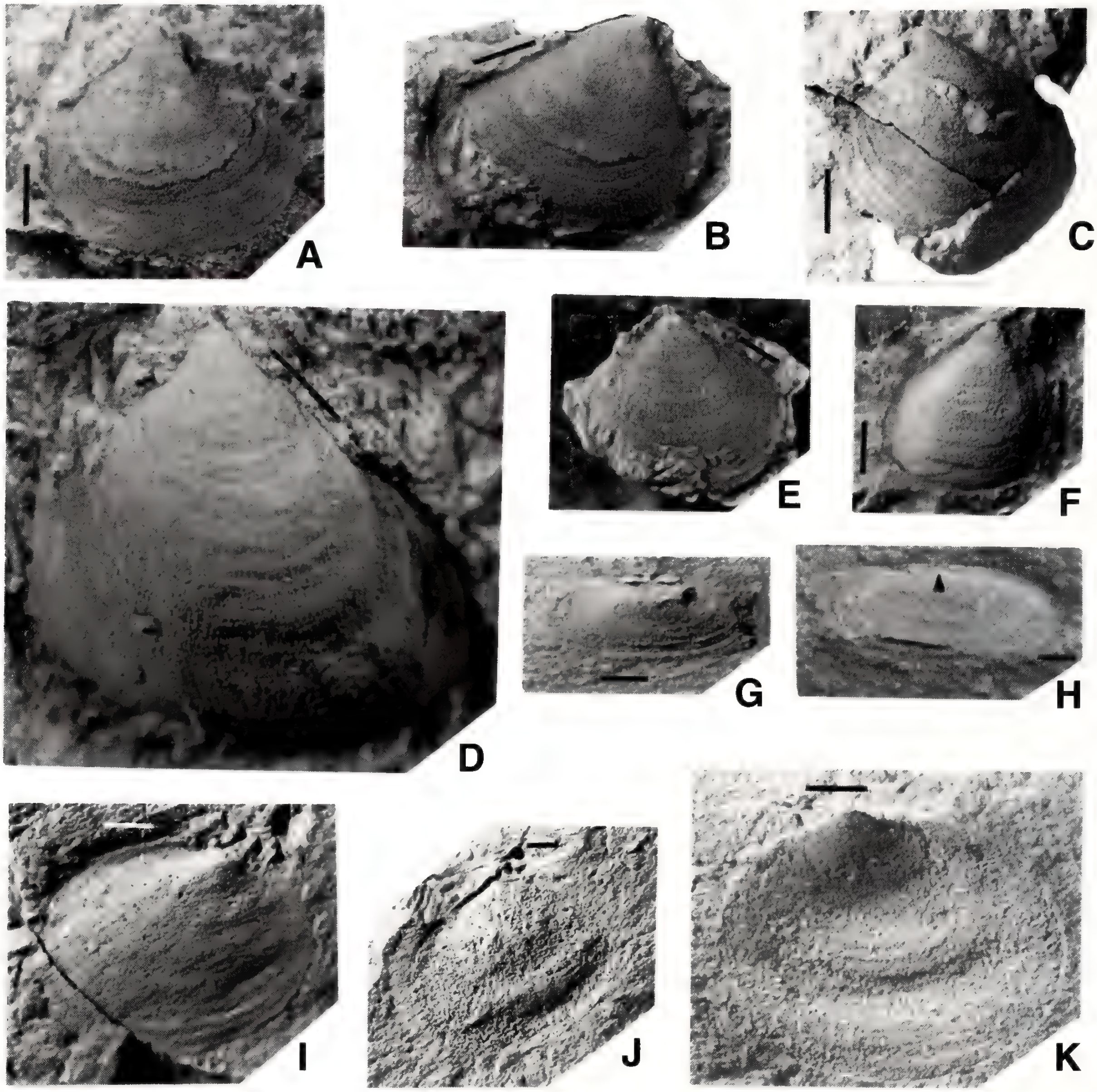

Fig. 3. Probable Lower Cambrian brachiopods that have been described as pelecypods. *Hubeinella formosa* Zhang (A-F), *"Davidia" dollfusi* Delgado (G), *"Modiolopsis" bocagei* Delgado (H), and *Xianfengoconcha minuta* Zhang (I-K). All scale bars represent 1 mm. **A,** paratype, figured by Zhang (1980a, pl. 3, fig. 3). **B,** paratype, figured by Zhang (1980a, pl. 3, fig. 4). **C,** paratype, figured by Zhang (1980a. pl. 3, fig. 2). **D,** holotype, figured by Zhang (1980a, pl. 3, fig. 1). **E,** previously unfigured specimen. **F,** paratype, figured by Zhang (1980a, pl. 3, fig. 5). A-F all from Lower Cambrian, China. **G, H,** symmetrical internal molds. Arrow points to apex of shell, which to either side has socket ridges. G and H are from Lower Cambrian, Portugal. **I,** holotype, figured by Zhang (1980a, pl. 2, figs. 12, 13). **J,** paratype, figured by Zhang (1980a, pl. 2, fig. 14). **K,** paratype, figured by Zhang (1980a, pl. 2, fig. 15). I-K are from Lower Cambrian rocks, China.

the Siberian Botomian Stage.

Subsequent to Zhang's work, Runnegar and Pojeta (1992) treated his taxa as stenothecoids, a group of uncertain affinities, and Geyer and Streng (1998:87) in discussing Zhang's taxa noted: "...the interpretation of the following taxa, all from the Early Cambrian of Hubei Province on the Cambrian Yangtze Platform, is refused: ... All of them are based on slightly or considerably distorted valves of inarticulate brachiopods rather than pelecypods."

A difficulty in using Zhang's work is the low printed quality of the photographic illustrations. I have had the opportunity to examine and photograph many of his specimens (Figs. 3A-F, I-K, 4, 5).

Hubeinella formosa Zhang (1980a, pl. 3, figs. 1-5;

1980b pl. 1, figs. 20-21; Fig. 3A-F herein), the type and only known species of *Hubeinella*, has a near teardrop shape and prominent comarginal ornament similar to stenothecoids (Horny, 1957; Koneva, 1976, 1979). However, such a shape also occurs in obolellid inarticulate brachiopods. Some specimens of *Hubeinella* (Fig. 3B, D, E) show faint radial external ornament, a feature that occurs in obolellids but is absent in stenothecoids (Yochelson, 1969). Unfortunately, nothing is known of the internal features of *Hubeinella*.

Of *Xianfengoconcha* Zhang, I was able to restudy and photograph three specimens of *X. minuta* Zhang (1980a, pl. 2, figs. 13-15; 1980b, Plate 1, figs. 13, 14; Figs. 3I-K, 4A herein). All three specimens are poorly preserved molds that show nothing beyond an inequilateral shape and faint coarse and fine comarginal ornament. *X. minuta* is one of three species placed in *Xianfengoconcha* by Zhang; his figures of *X. elliptica* Zhang, type species of the genus, and *X. rotunda* Zhang are no more informative than the specimens of *X. minuta*.

Zhang (1980a:10) provided a drawing of the hinge of *X. elliptica* that shows an elongate toothlike structure on each side of the beak. Such structures occur in obolellid brachiopods (Rowell, 1965:292-293) including *Lamellodonta* Vogel (Havlicek and Kriz, 1978). In any case, these teeth do not show well on Zhang's plate, and most of the specimens of *X. elliptica* and *X. rotunda* figured by him are essentially equilateral in shape.

The most that can be said about *Xianfengoconcha* is that the known specimens are of a bivalved creature that shows no diagnostic pelecypod features, and which probably is an inarticulate brachiopod.

I have been able to examine and photograph four specimens (including the holotype) of *Praelamellodonta elegansa* Zhang, the type species of *Praelamellodonta* (Zhang, 1980a, pl. 1, figs. 1, 3, 8, 12, 13; 1980b, pl. 1, figs. 1, 3, 5; Fig. 4B-H herein). The specimens range from a composite mold to one that is mostly covered with shell. The shape of the specimens varies as does the strength of the posterior umbonal slope, both of which suggest deformation of the original shape; the specimen shown in Figure 4H is clearly deformed.

The holotype of *Praelamellodonta elegansa* (Fig. 4D, E) has a prominent beak to either side of which is a bladelike structure, and at least three specimens preserve some radial ornament (Fig. 4C, G, H). The bladelike structures to either side of the beak and the presence of radial ornament suggest that the specimens of *P. elegansa* are distorted inarticulate brachiopods.

Thus, restudy of Zhang's specimens of *Hubeinella*, *Xianfengoconcha*, and *Praelamellodonta* reinforces the suggestion of Geyer and Streng (1998) that they are distorted valves of inarticulate brachiopods.

In addition to the above three genera, Zhang described the genus *Cycloconchoides* (Fig. 5A-D). He defined two species, *C. venustus* Zhang, the type species of the genus, and *C. elongatus* Zhang. I have examined and photographed four specimens including the holotypes of both species.

All the known specimens of *Cycloconchoides* preserve the shell and have a gross ornament of comarginal growth lines and radial ribs. No internal features are known. The growth lines are prominently raised and seem to truncate the radial ribs before the next set of ribs is formed. The general appearance of the specimens is that of a series of stacked shells such as occur in some arthropods that do not completely molt the old carapace. This is a prominent feature of conchostracan branchiopods some of which have radial ribs between the growth increments (Tasch, 1969). This type of ornament is not common in pelecypods and is not known to occur in any Cambrian or Ordovician species. However, branchiopods are not known from Lower Paleozoic rocks, and most branchiopods occur in fresh or brackish waters. Other bivalved arthropods are known from the Cambrian, such as *Canadaspis perfecta* (Walcott) (Conway Morris *et al.*, 1982); although *Canadaspis* does not stack its molts. Determining the affinities of *Cycloconchoides* will depend on finding new material that shows diagnostic features; specimens in which the shell is partially decorticated would be the most useful. It is unlikely that *Cycloconchoides* was either a pelecypod or a brachiopod.

MIDDLE AND UPPER CAMBRIAN TAXA

Recently, Middle Cambrian species of *Pojetaia* were described from Bornholm, Denmark (Hinz-Schallreuter, 1995) and Morocco (Geyer and Streng, 1998) (Fig. 2).

Geyer and Streng (1998) described the genus *Arhouriella* from the early Middle Cambrian Jbel Wawrmast Formation of Morocco (Fig. 6C). *A. opheodontoides* Geyer and Streng is known from two silicified incomplete presumed right valves. The species is roughly equilateral. Except for the hinge, internal features are poorly preserved; the authors (Geyer and Streng, 1998:93) noted that: "...the presence of a faint imprint interpreted as posterior adductor muscle scar [called anterior muscle scar in their fig. 7H]...Faint pallial [sic] commences at adductor muscle scar." These features are not readily apparent on their photographic figures and are not included on their reconstruction.

One specimen of *A. opheodontoides* preserves a well-developed slightly concave hinge plate and some of the presumed posterior ligament area, which partly covers

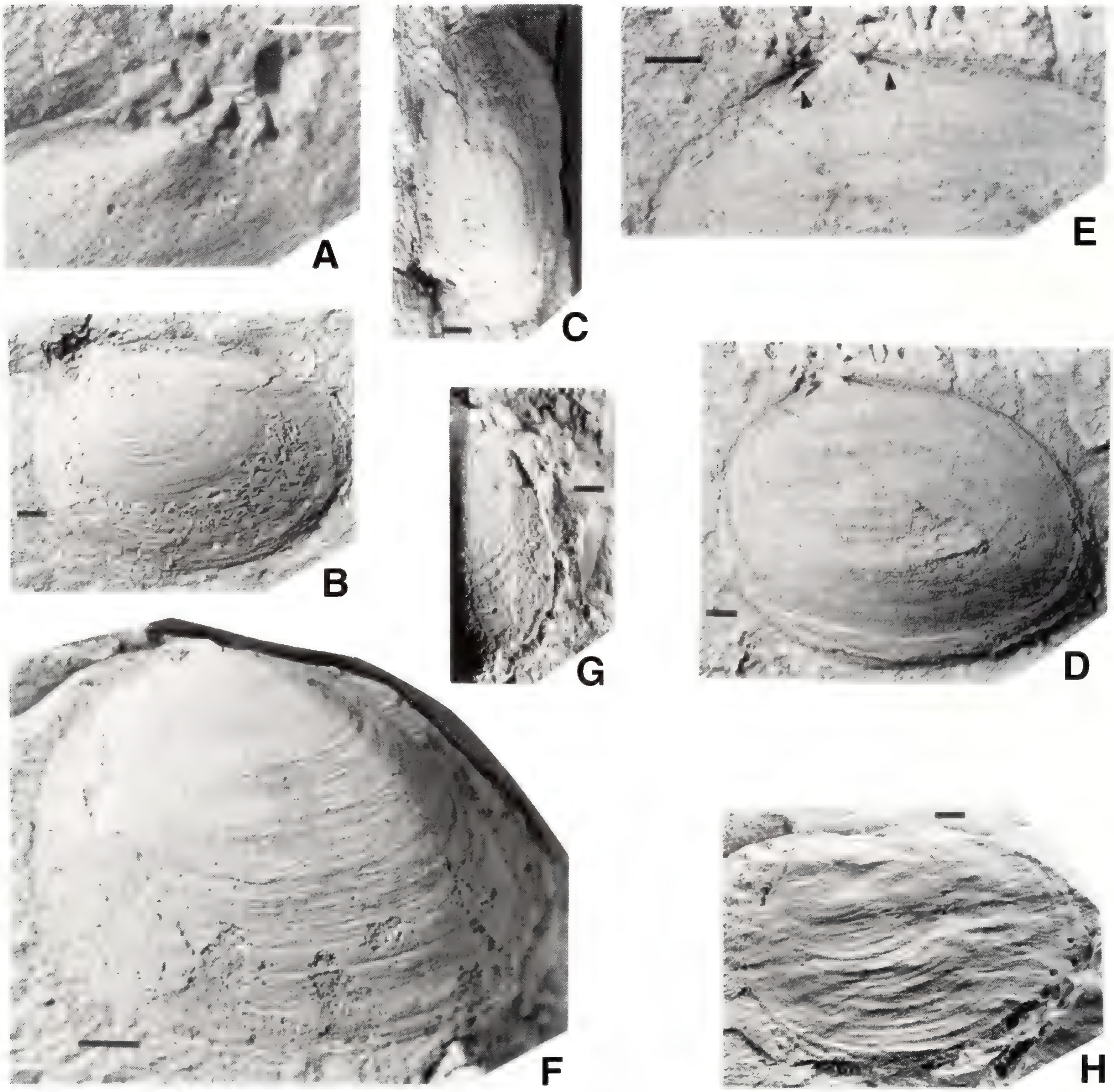

Fig. 4. Probable Lower Cambrian brachiopods from China that have been described as pelecypods. All bar scales represent 1 mm. *Xianfengoconcha minuta* Zhang (A) and *Praelamellodonta elegansa* Zhang (B-H). **A,** enlargement of apical area of specimen shown on Fig. 3I. **B, C,** paratype, C shows some radial ribbing, figured by Zhang (1980a, pl. 1, fig. 8). **D, E,** holotype, E is an enlargement of the apical area, arrows point to bladelike structures to either side of the apex, figured by Zhang (1980a, pl. 1, fig. 1). **F, G,** paratype, G shows some radial ribbing, figured by Zhang (1980a, pl. 1, figs. 12, 13). **H,** distorted paratype showing radial ribbing, figured by Zhang (1980a, pl. 2, fig. 3).

the hinge plate (Fig. 6C). The hinge plate undercuts the two pronglike central teeth. Anterior to the teeth there is an elongate fossette that Geyer and Streng interpret as an additional ligament area; they reckon that the ligament extended over the entire hinge plate in an early ontogenetic stage. The anterior of the two teeth originates directly on the dorsal margin and not on the hinge plate.

If *Arhouriella opheodontoides* is a pelecypod, and if the amphidetic interpretation of its ligament is correct, it is clearly very different from *Fordilla* and *Pojetaia* which have opisthodetic ligaments.

John C. W. Cope (pers. comm., September 1999) suggested that the teeth of *Arhouriella opheodontoides* could best be compared to those of the Upper Cretaceous solemyoid *Nucinella sohli* Pojeta (1988, pl. 5). Leaving aside the fact that the oldest known nucinellid is Early Jurassic in age, a major dentition difference between the two species is that in *N. sohli* the peglike teeth are mounted

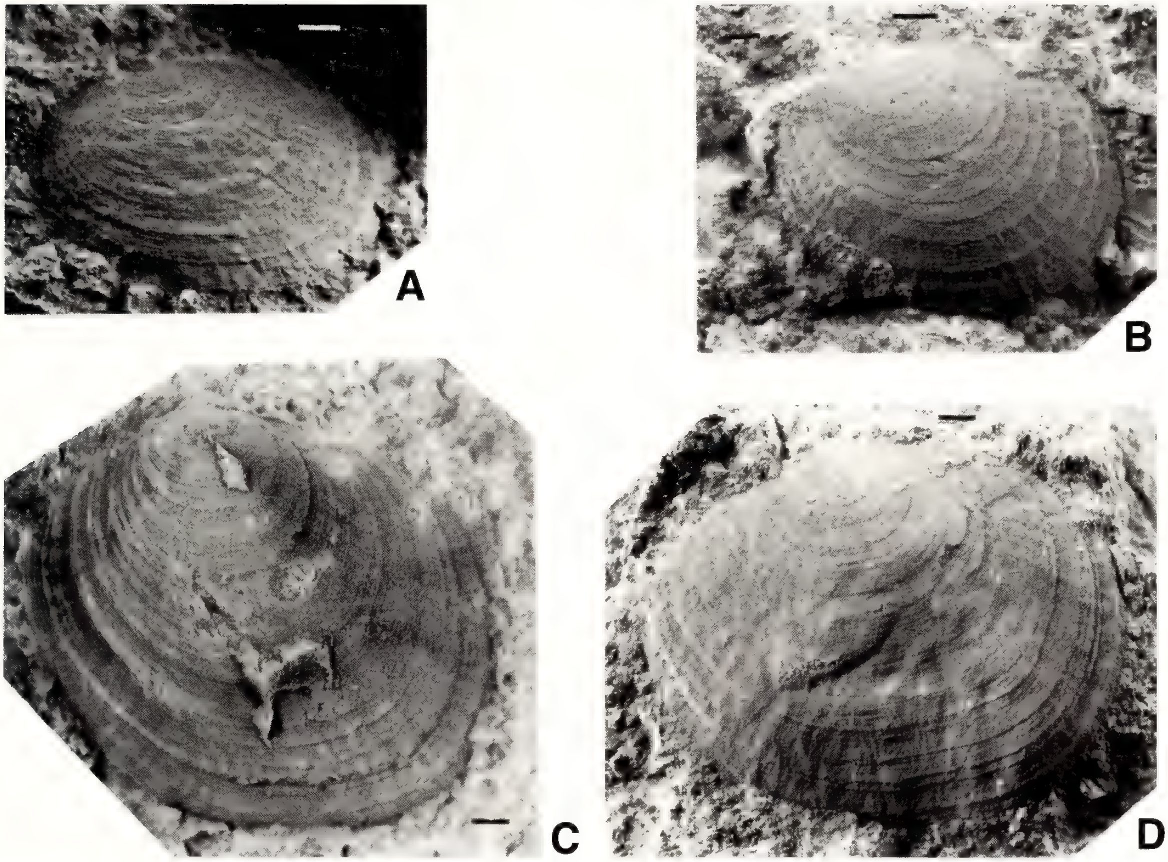

Fig. 5. *Incertae sedis* Lower Cambrian bivalves from China; possible arthropods. *Cycloconchoides elongatus* Zhang (A), and *C. venustus* Zhang (B-D). All scale bars represent 1 mm. **A,** holotype, figured by Zhang (1980a, pl. 3, fig. 14). **B,** paratype, figured by Zhang (1980a, pl. 3, fig. 8). **C,** paratype, figured by Zhang (1980a, pl. 3, fig. 9). **D,** holotype, figured by Zhang (1980a, pl. 3, fig. 6).

directly on the hinge plate, and, as in other solemyoids, *N. sohli* is anteriorly elongated.

Clearly, Geyer and Streng have a very unusual bivalve and additional specimens will help determine its systematic position.

Tuarangia paparua MacKinnon (1982) was first described from the Middle Cambrian Tasman Formation of New Zealand; the species is based on at least one hundred specimens. Subsequently, Berg-Madsen (1987) described *T. gravgaerdensis* from the Middle Cambrian Andrarum Limestone of Bornholm, Denmark.

Tuarangia (Fig. 6A, D-E) has been the subject of much discussion as to whether or not it is a pelecypod, and, if it is, how it is related to other pelecypods. MacKinnon (1982) placed *Tuarangia* in the Pteriomorphia with question, even though the genus possesses numerous taxodont teeth. He interpreted its shell microstructure as foliated calcite, which he noted in pelecypods occurs only in the Pteriomorphia. He also noted that the genus has an amphidetic ligament that shows as a raised ridge, between the two rows of taxodont teeth on internal molds.

Runnegar (1983) regarded *Tuarangia* as being related to the "quasirostroconch" *Pseudomyona queenslandica* (Runnegar and Jell), because both have foliated calcite shell microstructure, and he interpreted the presumed amphidetic ligament of *Tuarangia* as representing the place where a univalved protoconch had broken off from the rest of the shell. *Pseudomyona* is known to have a univalved protoconch and a bivalved adult shell. Runnegar thought of *Tuarangia* and *Pseudomyona* as bivalved monoplacophorans. Carter (1990:179) noted: "The order Tuarangioida is, therefore, presently regarded as a monoplacophoran derivative which is convergent toward the Bivalvia." Waller (1998:10) regarded Tuarangia as being poorly known and noted its various taxonomic placements.

With conviction, MacKinnon (1985) placed *Tuarangia* firmly in the Pteriomorphia and disassociated it from *Pseudomyona* by noting that the middorsal ridge on internal molds was the underside of an amphidetic ligament. The ridge between the rows of taxodont teeth is preserved in the same way on all known specimens, and he noted that there is no sign that it represents a broken-off

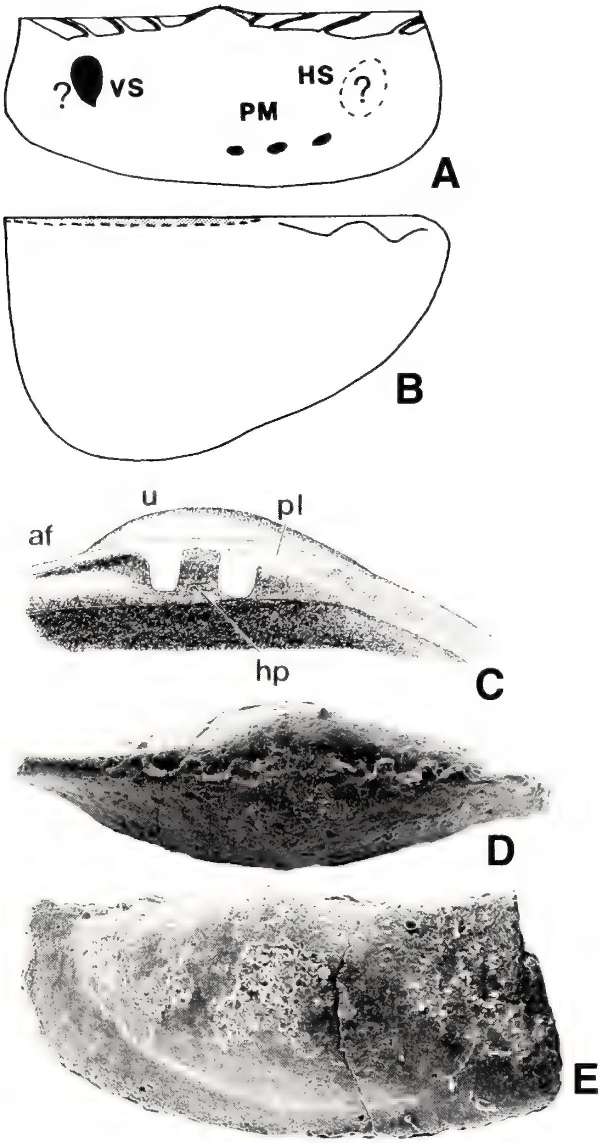

Fig. 6. Middle and Upper Cambrian pelecypods. *Tuarangia gravgaerdensis tenuiumbonata* (A), *Camya asy* (B), *Arhouriella opheodontoides* (C), and *Tuarangia paparua* (D, E). **A,** reconstruction of muscle scars from Hinz-Schallreuter (1995:75); VS = anterior adductor, HS = posterior adductor, PM = pallial line. **B,** drawing of shape and dentition from Hinz-Schallreuter (1995:75). **C,** drawing of hinge from Geyer and Streng (1998:93); af = anterior fossette, u = umbo, pl = posterior ligament area, hp = hinge plate. **D, E,** dorsal and left lateral views, specimen 0.9 mm long. Figures A and B reproduced with permission of *Geschiebekunde aktuell.* Figure C reproduced with permission of *Revista Española de Paleontologia.*

structure. Berg-Madsen (1987) maintained the placement of *Tuarangia* in the Pteriomorphia, but with reservations. She also figured *Tuarangia* sp. from an erratic boulder from Poland; the boulder contained Upper Cambrian conodonts, which indicate that *Tuarangia* ranged into lower Upper Cambrian. Runnegar and Pojeta (1992) maintained the close association of *Tuarangia* and *Pseudomyona*, based on similar shell microstructure, and separated them from the lineage including *Fordilla* and *Pojetaia*.

Hinz-Schallreuter (1995) described a pelecypod faunule from the Middle Cambrian Exsulans Limestone of Bornholm, Denmark. The faunule contains *Pojetaia ostseensis, Tuarangia gravgaerdensis tenuiumbonata*, and *Camya asy*, all named by Hinz-Schallreuter in her 1995 paper.

In discussing *Tuarangia gravgaerdensis tenuiumbonata*, Hinz-Schallreuter noted that she had complete shells showing both the interior and exterior and that they have a space for an amphidetic ligament as interpreted by MacKinnon (1982, 1985). Her description of this species noted the presence of pallial muscles and an anterior adductor muscle (Fig. 6A). These features do not show well where she notes their presence on her figure 5.3. The muscles are shown on her reconstruction (Hinz-Schallreuter, 1995:75) where the position of the anterior adductor muscle scar is in black and queried. She indicated the possible position of the posterior adductor muscle with a dashed open circle within which is a question mark; in her description she noted that the posterior adductor muscle was not preserved on her material. If Hinz-Schallreuter's interpretation of the musculature of *Tuarangia* proves correct, there is no doubt that it is a Cambrian pelecypod and is not related to *Pseudomyona*, because that genus has a single subcentral muscle. Cope and Babin (1999) accepted Hinz-Schallreuter's reconstruction of *Tuarangia.*

Hinz-Schallreuter also defined the Middle Cambrian pelecypod genus *Camya*, type species *C. asy*. This species (Fig. 6B) is like *Tuarangia* in its anterior-posterior elongation and long dorsal margin, but it is more constricted anteriorly; the beaks are in an anterior position, and there is a single tooth to either side of the beak. The species is known from two left valves that show comarginal growth lines. Other features of the species are unknown.

Babin (1993) has shown that the supposed Middle Cambrian pelecypod from Belgium, *Modiolopsis* ? *malaisii* Fraipont, 1910, is a pseudofossil.

Pojeta (1980:77) figured an unnamed possible Late Cambrian pelecypod from the Frederick Limestone of Maryland. This specimen shows only shell shape; no other specimens have been found, and its status as a pelecypod remains dubious.

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Ordovician to Devonian diversification of the Bivalvia

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Abstract: Studies of the Paleozoic Bivalvia have accelerated in the last three decades and we have numerous new data dealing with the diversification of this Class of mollusks. After the extreme scarcity of the Cambrian data, the abruptness of the diversity of the bivalve faunas in the fossil record during the Early Ordovician is an outstanding event. It is also noteworthy that both this first explosion and the succeeding diversification during the Middle Ordovician were located primarily on the Gondwanan and Avalonian shelves in shallow clastic facies. By contrast, from the late Ordovician, Baltica and Laurentia were more propitious for the further diversifications of bivalves, notably epibenthic ones. Thus before the close of the Period, all the subclasses of bivalves were established and the Class was dispersed throughout the world oceans. After the uppermost Ordovician extinctions, an important replacement at familial and generic levels occurred during the Silurian. The Pteriomorphia, many of them adapted to an epibyssate mode of life, underwent an explosive evolution, particularly during the Ludlow, while many free-burrowing suspension-feeding genera were adapted to the broad expanses of soft muds. During the Devonian, bivalves continued their diversification at both familial and generic levels, and for the first time, some of them colonized fresh-water. The continuing paleogeographic changes favored faunal exchanges, for example between Appalachian and western European areas, and led to the establishment of cosmopolitan faunas.

Key Words: bivalves, diversification, Paleozoic, Ordovician, Silurian, Devonian

"The evolutionary radiation of the bivalves is one of the success stories of invertebrate evolution; few other marine taxa have shown such a steady and consistent increase in diversity" (Gould, 1977:273). At present, the Class Bivalvia constitutes a major part of modern marine benthic faunas even without consideration of the freshwater representatives. This diversity of the bivalves (about 9000 living species according to Nicol, 1989) is the successful conclusion of a long history (Fig. 1), and it is interesting to attempt to track the key stages marking the beginning of this odyssey, especially the initial diversifications of the Class. The difficulties for such a process are numerous and any reconstruction is largely provisional. However in the three last decades, numerous new investigations have brought important data dealing with the Lower Paleozoic faunas of bivalves. Nevertheless, to estimate the value of the study of early diversifications we have to enumerate the main sources of difficulty.

MAIN DIFFICULTIES OF THE STUDY

• Systematics. Identifying the times of diversification requires expressing the succession of taxonomic diversities. For that, it is fitting to choose adequate taxonomic levels, obviously a subjective process. In previous papers (Babin, 1993a, 1995) I used genera and regrouped them at subclass level. I think that the results were conclusive enough but undoubtedly that procedure is not without problems.

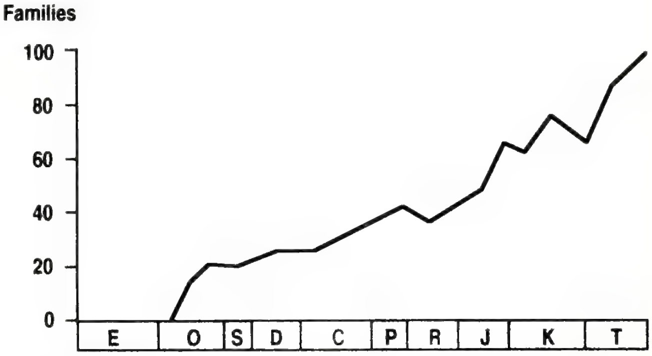

Fig. 1. The continuous increase of the diversity of the bivalves during the Phanerozoic. (after Paul, 1989).

In the literature, the genus concept of authors is probably only a little less insecure than the species one. The latter is the result of diverse splittings and regroupings. For instance, comparing Devonian faunas from Europe and North America, Bailey (1983) proposed a regrouping of 99 fossil species into only 16 species. Nevertheless, the notion of the genus is also sometimes the subject of dramatic changes. For instance, two classical genera in the old literature, the "praecardioids" *Cardiola* from the Silurian and *Buchiola* from the middle and upper Devonian have been split, the former into twelve genera (Kříž, 1979a), and the latter into six genera (Grimm, 1998).

Regarding the subclasses, there are also some differences of opinion amongst authors. The neontologists generally recognize two subclasses of Bivalvia, Protobranchia and Autobranchia, while paleontologists, basing their classification on the dentition, make use of more subclasses, considering them as evolutionary units. The latter are regarded rather as superorders in the former classification. Pojeta (1987) uses five subclasses: Palaeotaxodonta, Isofilibranchia, Heteroconchia, Pteriomorphia and Anomalodesmata. Cope (1995) defines seven subclasses: Palaeotaxodonta, Lipodonta (for the single solemyoids included in the Cryptodonta by Newell, 1969 and in the Palaeotaxodonta by Pojeta, 1988), Palaeoheterodonta (including the mytiloids which are Isofilibranchia for Pojeta), Heterodonta (these two units are included in Heteroconchia by Pojeta), Neotaxodonta, Pteriomorphia and Anomalodesmata. We retained Cope's classification in a recent paper about the Ordovician diversification (Cope and Babin, 1999); nevertheless, Cope (1998) recently proposed that "some long-accepted palaeoheterodonts are in fact heterodonts, and that even in the early Ordovician heterodonts were already present." In these works all extant bivalve subclasses were represented from the early Ordovician. However, another subclass, never mentioned in all these papers, is the Cryptodonta, which included (Newell, 1969) two orders, solemyoids and praecardioids. The former are possibly paleotaxodonts or lipodonts (see above) but the latter raise a true problem. They were abundant during the Paleozoic, particularly during the Silurian; Kříž (1965, 1979) has put a large part of them into the isofilibranchs and the pteriomorph arcoids (which would be neotaxodonts for Cope) but he has lately kept Cryptodonta for the praecardiids and antipleurids (Kříž, 1996). More recently, Johnston and Collom (1998) have used afresh the subclass Cryptodonta for numerous bivalves. It is not an unimportant choice regarding the evolution of the bivalves because Cryptodonta, if they include only the praecardioids, would be the single subclass of Bivalvia without modern representatives.

Last but not the least, it is evident that these problems of classification also reflect different conceptions about the phylogeny of the bivalves (see for instance, Cope, 1995; Waller, 1998). Notwithstanding their importance, these points are beyond the present enquiry and they are not really constraining for my purpose. So, despite these undeniable divergences and difficulties, it remains possible to appraise the properties of the initial bivalve diversifications.

- Fossil determinations correspond to another problem of taxonomy. Paleontological material is often of poor preservation. Paleozoic bivalve fossils are often poor molds showing few characters without the dentition for instance. So many determinations have been based solely on shell shape explaining many "wastebasket" taxa; in the older literature genera such as *Ctenodonta* or *Modiomorpha* can appear to have had a very large spatio-temporal distribution. It is another cause of weakness for the inventory in addition to the splitting or regrouping cited above.

- Field investigations and study of the new material are of course among the most important factors initiating change in diversity estimates. The comparison of recent graphs of the Ordovician bivalve diversification, for example, shows an important amplification of the generic numbers for South Gondwana and Avalonia (Fig. 2). It is the result of new data from Argentina on the one hand (Sánchez, 1999), and from Wales on the other (Cope, 1996).

Another example may be given. There is no paleotaxodont in my database for the middle Ordovician from Baltica. However, in a recent paper Dzik (1994, Fig. 24, A-C) figured a minute specimen of a juvenile paleotaxodont bivalve (*Praenucula*?) from the lower Llanvirn of Baltica.

- Chronology. Identifying the times of diversification requires having a sufficient resolution of the geologic time scale and precise chronologic correlations between the different parts of the Earth. The stratigraphic information concerning the lower Paleozoic is indeed various and of irregular quality according to the regions. In addition there are several regional scales and they often remain without clear correlation. In this way, I have underlined (Babin, 1995), for example, the equivocal use of middle Ordovician in the North American literature compared with the standard scale of Great Britain. The American Blackriveran stage, containing bivalves, considered as middle Ordovician corresponds really to the lower part of the Caradoc Series from the upper Ordovician in the standard scale. On that basis, we have not known until now bivalves from the middle Ordovician in North America.

Elsewhere, in regions where stratigraphical investigations are incomplete, we can sometimes read Ordovician, Silurian or Devonian without more precision and we are hazy about the times. In the *Treatise on Invertebrate Paleontology* (1969), such vagueness is common enough too, even though it has been the reference for several published compilations.

To establish the database one must evidently consult the old fundamental publications of the last century. Unfortunately, the stratigraphical location of the fossils, which are described and figured, is usually imprecise. Barrande (1881) published the volume VI of his monumental "Système silurien du centre de la Bohême." Devoted to

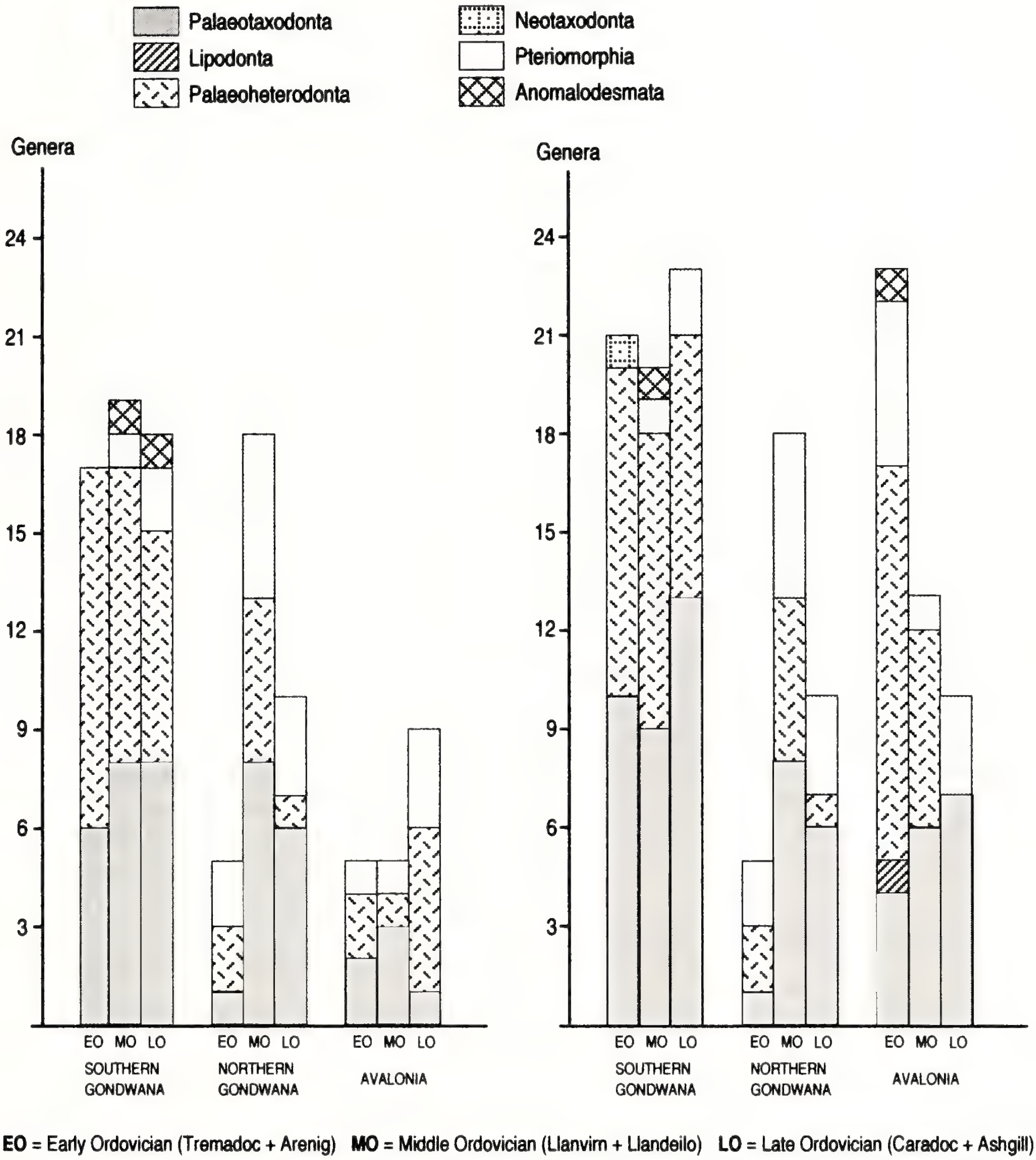

Fig. 2. Comparison of two successive compilations of data concerning the Ordovician bivalves. It shows the effect of the recent investigations in the field. Left: data from 1993 (after Babin). Right: data from 1999.

the "Acéphalés" (*i.e.* to the bivalves), it comprises 342 pages and 361 plates to describe 1269 species distributed among 58 genera, of which 20 are new. However, they are not all from the Silurian, as the title mentions, but they are distributed from the lower Ordovician (Llanvirn) to the middle Devonian (Givetian) because Barrande always used Murchison's original definition (1835) of the Silurian Period. Thus, to utilize Barrande's data, one needs to refer to more recent documents of Czech geologists for the temporal correlations. Concerning the Devonian, the important books of Hall (1884, 1885) with the description of 69 genera, of which 49 were established in North America, and of Beushausen (1895) with the description of 46 genera from the Rhineland, excluding the pteriomorphs studied by Frech (1891), are easier to consult regarding the stratigraphical assignment. Nevertheless, the utilization of the data for the database requires the establishing of synonymies between all these works.

These non-exhaustive remarks go to show that every database is imperfect, provisional and could be continually improved.

- Paleogeography. To locate the paleodiversifications in space we have at our disposal numerous and more or less different paleogeographic reconstructions. Despite great strides over three decades in this field following the plate tectonics model, we know that these reconstructions remain difficult for the Paleozoic. However, a general enough agreement exists today about the broad outlines of the relative position of the large paleocontinents and it is sufficient for the present matter.

Taking all these restrictions into consideration, I will consider the available data to draw the main events regarding bivalve paleodiversifications from the Ordovician to the Devonian. Nevertheless, before examining the rough data, diversifications need some consideration. Actually, when the radiations are put in evidence, the question remains of their explanation. Two categories of causes need consideration. Are the diversifications the result of intrinsic factors or of inductive extrinsic conditions? The former may particularly correspond to key innovations and it is understandable that many new structures, innovative ones, are not preserved in the fossil record. The interpretation of the material, more often than not hypothetical, will be an inescapable way to reconstruct the soft parts. As for the extrinsic factors, some of them are given by the sediments like the grain-size or some information regarding the bathymetry, the oxygenation, etc., but many other factors will remain hypothetical too.

THE GREAT ORDOVICIAN RADIATION

The need to look at the Ordovician diversification has been renewed for some years because this Period encompasses one of the most important evolutionary radiations. This is particularly clear regarding the bivalves, which offer in the fossil record a true explosion during the Arenig (late Lower Ordovician). In reality, there is a very poor record and important gaps in the story of Bivalvia before the Arenig and one may consider that this apparent explosive diversification is an artefact. The extremely scarce representatives of Cambrian bivalves, small shells of perhaps meiofauna and "the abysmally poor record of bivalves from the Tremadoc Series (early Lower Ordovician) of the Ordovician" (Cope, 1995) suggest such an artefact. Diverse reasons can be offered to explain this lack of fossil bivalves, such as their particular fragility, unfavorable environmental conditions for their preservation, or geodynamical causes such as the rifting of the near-shore shelves that they inhabited, or even deficiency of the investigations, among others. No one consideration is really convincing. There are fossils of other marine benthic groups during the upper Cambrian and Tremadoc. Finally, as the Ordovician was a period of important radiation for many groups, it is not surprising to observe the same for bivalves. However, its explosive aspect with the simultaneous appearance of representatives of each subclass is very peculiar. Unfortunately, the paucity of information before the Arenig remains a serious handicap to understanding the temporal relations between the subclasses.

Considering the known fossil record, it is noticeable that these Arenig bivalve faunas were located on the peri-Gondwanan shelves (Babin, 1993b), Avalonia being considered as a neighboring area (Fig. 3). Possible reasons to explain this spatial restriction have been considered (Babin, 1995, Cope and Babin, 1999). Clearly the temperature of the sea water was not a deciding factor since the faunas were settled from very high latitudes (Morocco, Montagne Noire) to very low latitudes (Argentina, Australia), in spite of some obvious preferences such as the deposit-feeding paleotaxodonts, for instance, which are more numerous and varied in the high latitudes. On the other hand, a condition shared by the whole peri-Gondwanan shelves is siliciclastic sedimentation, and it is clear that all these first bivalves inhabited clastic sea-beds wherein they had infaunal or semi-infaunal modes of life. This relationship was probably linked with food supply because terrigenous sediments are considered more favorable from this point of view. Moreover, another distinctive feature of the Early Ordovician bivalve settings is their shallowness. The deep-

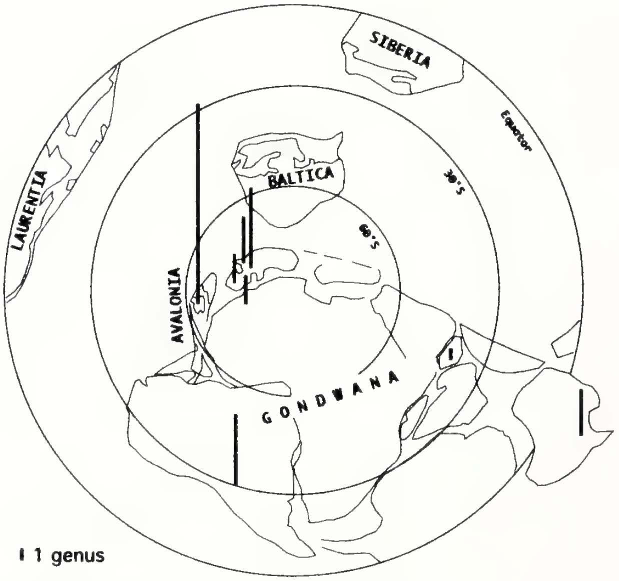

Fig. 3. Peri-Gondwanan location of the bivalves during the late Tremadoc-Arenig, south polar view.

est environments were perhaps those of the Montagne Noire (France), but it was certainly less than fifty meters in depth. So, it is clear that the early bivalve diversification was globally located in relatively shallow waters and is in accordance with Jablonski and Bottjer's model (1990) regarding the role of the onshore settings for the appearance of the major groups. In these environments, the empty ecospace hypothesis (Erwin, 1994) may help explain the burst of the endobenthic and semi-infaunal bivalves, which were the first representatives of shelly burrowers.

Finally, it seems that this preference for siliciclastic sedimentation may have been the restrictive factor against a geographic expansion towards other plate margins like Baltica or Laurentia [the recent assertion of Droser *et al.* (1995) and of Droser *et al.* (1996) that "bivalves occur in Ibexian strata" in shell beds is probably erroneous. The oldest North American described bivalves are from the uppermost Whiterockian or very lowest Blackriveran (pers. comm. of R. Ross, 4 Oct. 1999)]. It is also plausible that sheer ocean width was a barrier to the migration of the planktic larvae. Bivalve larvae live about six weeks before settlement; however Baltica, for instance, was not so far from Gondwana-Avalonia (Fig. 3). Prevailing surface currents could have been unfavorable but other benthic Arenig mollusks like rostroconchs were nevertheless cosmopolitan.

The other aspect to consider about the initial diversification concerns the possible impact of some intrinsic factor. I have said that it is difficult with the fossil material, that is with the hard parts (shells) only, to discern one or several key characters that induced the adaptive radiation. Stanley (1975, 1977) suggested that "the delayed radiation (since the first Cambrian bivalves) was triggered by the origin of the byssus" and that "retention of the byssus into the adult stage was an important aspect of the initial adaptive radiation of the class." However, this consideration seems based on North American bivalves with emphasis on the upper Ordovician faunas with pteriomorphs. Tevesz and McCall (1976, 1985) who proposed an epifaunal mode of life for Cambrian bivalves, suggested "that the Ordovician expansion of pelecypods is due to their invasion of the infauna habitat" and "that the delayed radiation of pelecypods was due to the Ordovician evolution of some other non-preserved structure that promoted survival of the group, such as, for example, some essential pedal musculature or a more efficient circulatory system."

One fundamental anatomical change could have been realized with the development of gills involved in the feeding process, that is with the appearance of the filibranch gill (Yonge 1947). Waller (1988) suggested that the *Tironucula* group, small paleotaxodonts from the Llanvirn (Middle Ordovician; see below), "probably had already evolved filibranchiate gills", and since 1995 Cope has correlated this change of the gills with the early diversification of the bivalves. Cope also looked for paleotaxodonts that could have acquired this important novelty. By analysis of the dentition allowing rapid fluttering of the valves to void the pseudofaeces, Cope (1995) proposed that it could have been the *Cardiolaria* group (Fig. 4). The advantages and changes of the mode of life resulting from this key innovation have been discussed elsewhere (Cope and Babin, 1999).

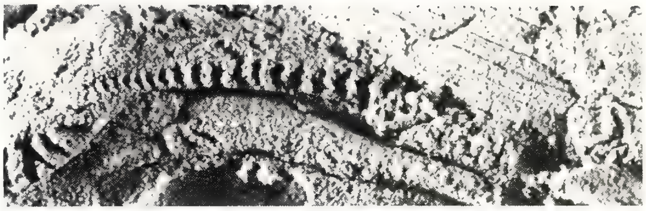

Fig. 4. *Cardiolaria beirensis* (Sharpe, 1853) from the mid Ordovician of Spain. This paleotaxodont shows two separate series of teeth anterior and posterior; Cope (1995) assumed that this arrangement could be functionally related with filibranch gills. x6.

However that may be, it appears that the Arenig bivalve faunas, located in Gondwana and Avalonia, contained representatives of all subclasses except Cryptodonta, if that is restricted to the praecardioids. The causes of this explosive radiation remain unclear, probably an interference of intrinsic and extrinsic factors, but the first unknown divergences took place probably before the Arenig (five or six genera are known from the latest Tremadoc of Gondwana).

During the Middle Ordovician, that is the Llanvirn of the standard stratigraphical scale, bivalve faunas remain located in essentially the same areas, often with a great abundance and variety such as in the Ibero-Armorican massif (Babin and Gutiérrez-Marco, 1991). Seven genera are also known in Avalonia (Cope, 1999). The Llanvirn was a period of transgression, and the flooding of the Gondwana craton also stimulated the burst of other groups like the trilobites. However, the geodynamic activity simultaneously modified the relations between the continental plates; thus, the progressive reduction of the Iapetus Ocean allows some communication between both its sides (Fortey and Cocks, 1988). For the first time we find some scarce bivalves in Baltica with *Babinka* and in Laurentia (Svalbard) with *Tironucula*.

A large dispersal of the bivalves over an extensive area (Baltica, Laurentia, Siberia) took place during the Upper Ordovician (Fig. 3) probably favored by the mid-Caradoc transgression, perhaps the most extensive in Phanerozoic history. The Chinese Ordovician bivalves probably proceed too from the upper Ordovician. It is no doubt the case for some of the genera in Yunnan cited by

Guo (1985) and assigned possibly to the Arenig-Llanvirn. Then, the dispersal in the earliest Late Ordovician provides bivalves firstly in the inshore siliciclastic environments such as the St. Peter Sandstone (Minnesota) but it corresponds rapidly to a second important diversification exploring particularly the epibenthic mode of life on the favorable carbonate platforms of these low latitudes. The pteriomorphs, for instance, at the time rapidly radiate while some modiomorphoids (*Modiolopsis, Corallidomus*, and *Whiteavesia*) are the first gregarious genera. Are Cryptodonta also present in the Late Ordovician of Laurentia? A species of *Vlasta (V. americana)* was cited by Fritz (1951) in the Richmondian of Canada but Kříž (1998) considers it is not a *Vlasta*. Elsewhere, other genera of the Upper Ordovician, such as *Shanina* from China and *Shaninopsis* from Sweden are not Vlastidae after Kříž (1998). We do not know precisely whether there were Cryptodonta in the Upper Ordovician. The origin of this subclass is obscure.

Simultaneously, in the high latitudes of south Gondwana (Ibero-Armorican area, Bohemia) bivalves became scarce and we do not know the reason. It is long before the cooling of the latest Ordovician and in any case some bivalves continue during the Ashgill in Morocco near the Gondwanan cap-ice.

Thus, the major paleodiversification of bivalves took place on the Gondwanan and neighboring Avalonian shelves as early as Late Tremadoc-Early Arenig. The dispersal and migration during the Late Ordovician favored particularly the radiation of epifaunal bivalves. The first peak of the diversity of the suspension feeding bivalves took place throughout the Upper Ordovician (Bretsky, 1973).

It is noticeable also that the sizes of the first bivalve representatives were small or moderate, the largest known form is a *Modiolopsis* from the Arenig of Wales of almost 50 mm length (Cope, 1996). The increase of size in the Middle and Upper Ordovician affected especially the pteriomorphs and paleoheterodonts but the largest bivalve known from the Ordovician is the problematic *"Vlasta" americana* quoted above with a length of about 120 mm (Fritz, 1951).

THE SILURIAN - DOWNTURN FOR THE BIVALVES

The mass extinction of the latest Ordovician, probably one of the most drastic crises, evidently affected the bivalves; nevertheless all the subclasses crossed the threshold of the Silurian. Moreover it is noteworthy that the Bivalvia have stood up to all the mass extinctions (Fig. 1), and their better success than other benthic groups (brachiopods for instance) is often explained by this remarkable adaptive resistance to major crises.

In a fundamental paper Kříž (1984) gave a quantitative overview of changes in bivalve faunas between the Ordovician and Silurian and of the new Silurian radiation of the Class. He worked with data taken from the *Treatise on Invertebrate Paleontology* (1969) modified by his own taxonomic concepts classifying, for instance, a large part of the Cryptodonta of the *Treatise* in the pteriomorphs. In spite of the new data gathered since 1984, this analysis is still probably conclusive enough. It shows that about 30% of the Ordovician genera survived into the Silurian; 35% of them were free-burrowers and 58% were endobyssate infaunal and semi-infaunal. Obviously, the epifaunal bivalves were less resistant against this crisis from which the causes (climatic deterioration, regression then transgression with anoxic conditions) are probably many and are poorly established. The widespread anoxic conditions persisted during the Early Silurian (Llandovery) and it was only a period of survival for the bivalves without clear recovery (Fig. 5). Among the fauna, the paleotaxodonts included several surviving Ordovician genera (about eleven after Liljedahl, 1994), but their diversity had distinctly decreased; they were widely distributed but with a possible preference for the equatorial areas (Laurentia, Baltica, South China, Australia). However, they were sometimes present at higher latitudes (South America; Sánchez *et al.*, 1995). Locally, as in Gotland (Baltica) some endemic genera appeared (Liljedahl, 1984). However, the subclass did not experience Silurian expansion. The paleoheterodonts, anomalodesmatans, some pteriomorphs, endobyssate infaunal and semi-infaunal suspension feeders, were the major participants of a very weak diversity increase while the epifaunal pteriomorphs remained inconspicuous after an almost total extinction at the end of the Ordovician (Fig. 5).

The environmental changes of the Middle Silurian (Wenlock) induced a new bivalve diversification which continued during the Ludlow (early Late Silurian). In several basins (Bohemia, Montagne Noire, Morocco, etc.), the development of alternating soft muds and biodetrital deposits favored the abrupt diversification of shallow free-burrowing suspension-feeders. Following Kříž (1979), the new specialized genera, such as *Cardiola* (Fig. 9) and others that he considers as pteriomorphs (see above), proceeded from epi- or endobyssate ancestors by a reversion neoteny. In these environments endobyssate infaunal and endobyssate semi-infaunal suspension-feeders also showed a rapid development with, for instance, Modiomorphidae and some (?) Pterineidae [Stanley (1972) and Bailey (1983) admitted that some Paleozoic pterineids were endobyssate; for Johnston (1993), these views are poorly supported, and all the pterineids were epibyssate]. Lastly, the epibyssate

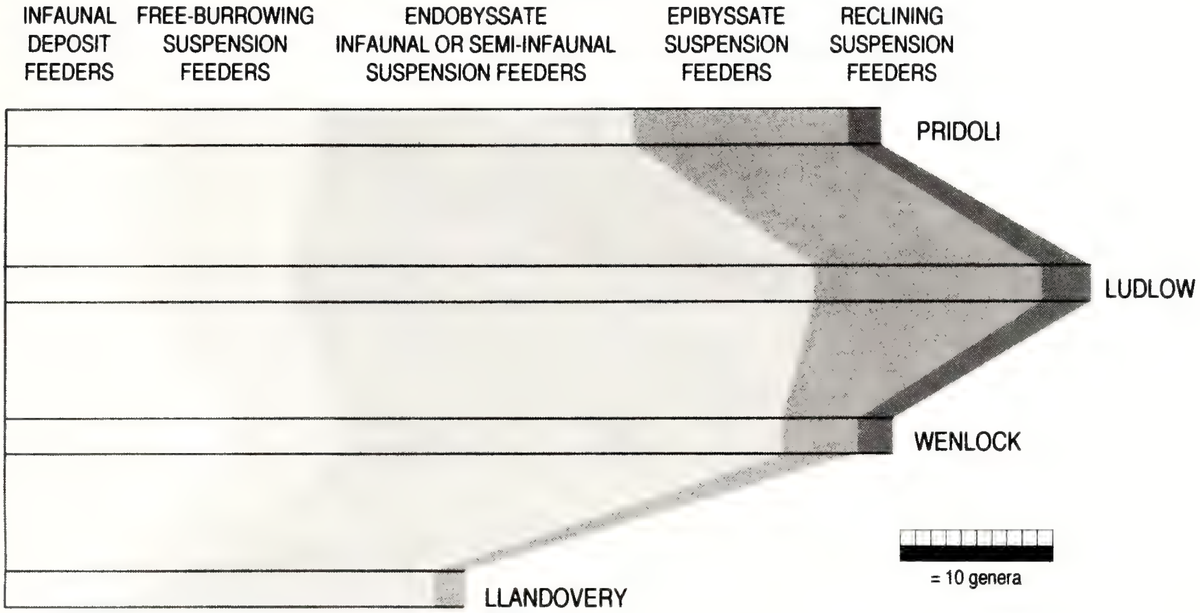

Fig. 5. Silurian diversification of the bivalves with their life habits (after Kříž, 1984, modified).

bivalves increased rapidly after the Wenlock and during the Late Silurian, and Kříž (1984) considers that this extensive adaptation is "one of the most important characteristics of the evolution of the Bivalvia in the Period." The distribution of the Bohemian-like fauna, linked with low oxygen conditions on the sea-bed is explained by the surface current circulation (Bogolepova and Kříž, 1995). Johnston and Collom's new ideas on cryptodonts as chemoautotrophs (1998) are supported by the existence of these low oxygen conditions. It is interesting to note that in their paper, Bogolepova and Kříž (1995) indicate the presence of such fossils as early as the lower Llandovery (basal Silurian) in Siberia, considering them as ancestral forms of the Bohemian-type bivalves. Can we regard the Siberian area as the cradle of these forms? It is not possible to reply with certainty to such a question. To locate precisely the cradles of the groups remains another problem generally insoluble for the paleodiversifications. During the Wenlock and Ludlow, some rare elements of the Bohemian-type fauna could have reached other areas, *Slava* is known in Laurentia (Pojeta and Norford, 1987), *Dualina* is cited in South America (Sánchez, 1991) and Florida (Pojeta *et al.*, 1976).

The fauna considered in these above-mentioned basins is different from that of the other areas dominated by paleotaxodonts, paleoheterodonts, and anomalodesmatans (Fig. 6). In these associations occurring in both clastic and carbonate platform muds, deposit-feeding species (paleotaxodonts) generally formed a large majority of the bivalves. Lastly, in the reef communities developed in several areas the bivalves constituted a minor group (Watkins, 1997). They were represented especially by epibyssate suspension-feeders like ambonychiids, pterineids, with genera known in other level-bottom associations with a lower species diversity and with larger specimen sizes. Apart from the megalodont *Megalomoidea* known in some reefs, there were no bivalves specialized for reef environments.

Finally, succeeding the Late Ordovician mass extinction, the Silurian was a Period of progressive recovery for the bivalves. Two major kinds of environments induced different adaptive responses. On the platforms, with normally oxygenated waters and at all latitudes, representatives of the main subclasses are found. In the Bohemian-type basins, environmental conditions with a low oxygenation induced an explosive evolution of the Pteriomorphia and Cryptodonta giving an odd cachet to the faunas. A slight decrease in bivalve diversity occurred during the Pridoli (Late Silurian); however, there was not a crisis like that of the Upper Ordovician.

THE DEVONIAN - DIVERSIFICATION AND DISPERSAL OF BIVALVE FAUNAS

The Devonian was another period of important diversification for the bivalves. According to Kříž (1979b), basing his argument on the *Treatise on Invertebrate Paleontology* (1969), the Devonian diversity in comparison with that of the Silurian increased 59% for genera and 36% for families. Amongst the latter it is noticeable that a dozen

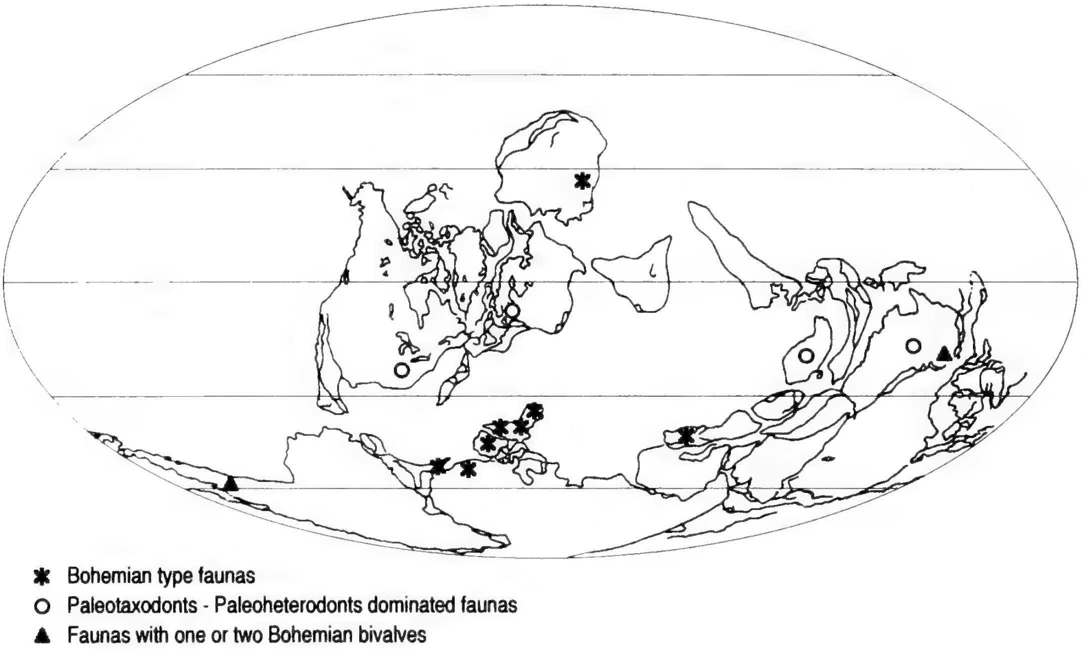
✱ Bohemian type faunas
○ Paleotaxodonts - Paleoheterodonts dominated faunas
▲ Faunas with one or two Bohemian bivalves

Fig. 6. Distribution of Wenlockian bivalve faunas (after Babin, 1993a, updated).

of them are extant families, four of which are new families of Heterodonta. Thus the Devonian bivalve fauna acquired a more modern aspect.

The increase in diversity was progressive during the Early Devonian and attained a peak in the Emsian. A first decrease took place during the Middle Devonian (Fig. 7) with different aspects according to the regions. After the Givetian, the global decrease became more pronounced until the end of the Devonian. It may be the result of "plate-tectonic regulation" (Kříž, 1979b) but it was also probably related to the development of regional environmental deterioration, especially in regard to benthic oxygen.

The Early Devonian bivalve faunas were particularly numerous on the terrigenous shallow sea-floors. The paleotaxodonts remained abundant in such environments and sometimes associations of *Palaeoneilo* or *Nuculites* were almost monospecific. Nevertheless, the pteriomorphs and the anomalodesmatans were particularly varied. Some of the former were possibly attached to flexible algae (Johnston, 1993) though the largest of them were fixed on elements on the sea-bed. In Europe, the Lower Devonian bivalves have been described and listed since the nineteenth century in the siliciclastic sediments of the Rhenish area (Frech, 1891; Beushausen, 1895; Maillieux, 1937, etc.) where they were especially numerous, a proportion of them being endemic genera. Similarly, in the carbonate facies of Bohemia a large part of the bivalve genera were endemic such as the cryptodonts *Antipleura* or *Kralovna*. In North America the generic variety is lesser but recent investigations (Johnston and Goodbody, 1988; Desbiens, 1994a, b) show, in siliciclastic sediments of Canada, the presence of several Rhenish genera. In South America in cool water the genera cited (Sánchez *et al.,* 1995) were cosmopolitan. In North China, 28 genera are cited, all of which were cosmopolitan, whereas 13 of the 56 Lower Devonian genera cited in South China were endemic, especially during the

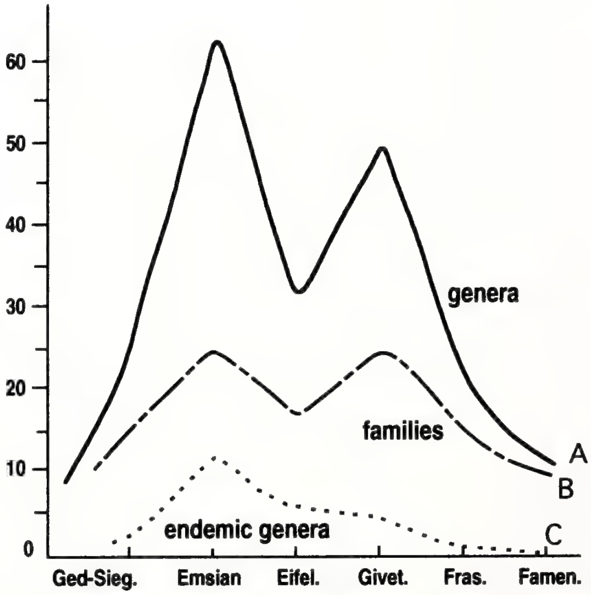

Fig. 7. Curves showing variations of Devonian bivalves in China. The Y-axis is Number (after Zhang, 1988).

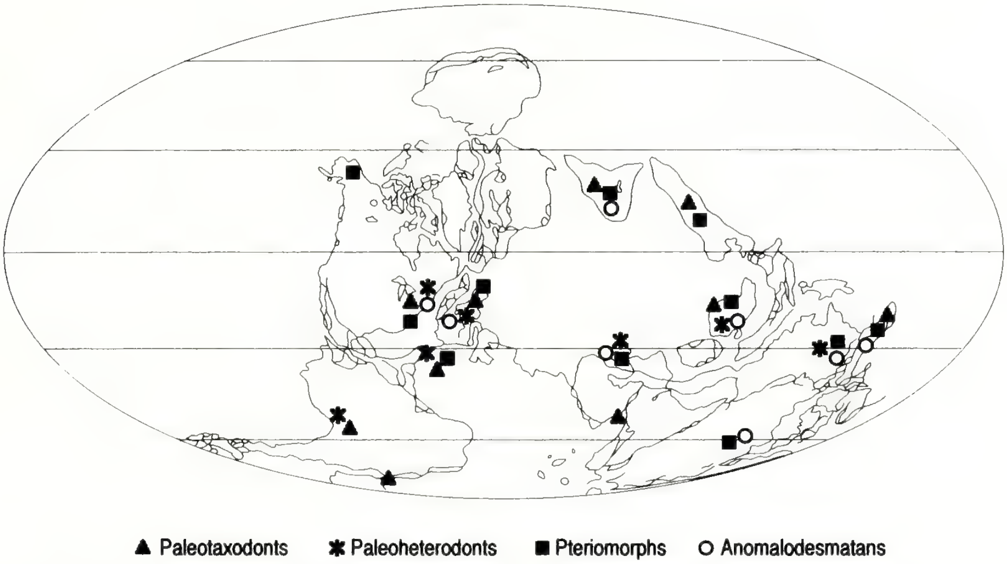

Fig. 8. Characteristics of Emsian bivalve faunas. West-European faunas have been taken as reference, and for each region those groups are shown that have at least three genera in common with them (after Babin, 1993a, updated).

Emsian (Zhang, 1988). Like the Rhenish area, the latter region was apparently an important center of diversification (Fig. 8). In southeastern Australia, Chapman (1908) described 27 genera and noted that they were known in North America or Europe. More recently, from a shallow marine carbonate shelf environment Johnston (1993) described a remarkably preserved silicified fauna of bivalves. Amongst 24 genera cited, two or three are endemic; the others show close affinities with the bivalves of central Europe. From the lower Devonian (probably Emsian) of New Zealand, Bradshaw (1999) lists 27 genera and she acknowledges only "a distant link with Europe" for her fauna, however at the generic level, at least 16 of these taxa are known also in Europe. Finally, bivalves experienced a noteworthy diversification during Emsian times but they evolved only at family, genus and species levels. This radiation is probably related to the Emsian transgressions, and the most favorable environments seem have been shallow terrigenous shelves located at low latitudes such as central Europe or south China.

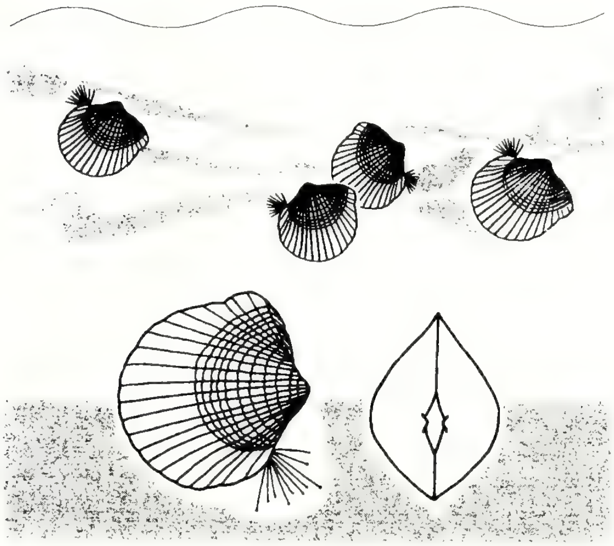

Fig. 9. Possible life habits of small praecardioids. *Cardiola* (below; Silurian) as semi-endobyssate according to Kříž (1979a). *Buchiola* (above; upper Devonian) as epibyssate pseudoplankton according to Babin (1966) (after Vannier *et al.*, 1995; modified).

After the Emsian acme a slight decline occurred during the Middle Devonian. However bivalve faunas were still flourishing in many regions but with little new radiation. For instance, the megalodontids show some adaptive diversification with new genera such as *Megalodon* and *Eomegalodus*, these having a thick shell related to coral environments. The most significant for Middle Devonian times is the continued dispersal of the bivalve faunas, resulting in increased cosmopolitanism. It is the result of the progressive restriction of some oceanic areas such as the Rheic between Europe and North America. Bailey (1983) has finely illustrated the similarities between the Rhenish and Appalachian communities; however, there was some diachronism, since the Middle Devonian faunas of North America include Lower Devonian forms from Western Europe. Therefore, the fauna migrated westwards giving a large expansion to the genera initiated primarily in the Rhineland.

The decline of the bivalve faunas continued through the Late Devonian, but the paleogeographic changes induced some new limited diversifications. For instance, the development of large continental areas such as the Red Sandstone continent was favorable to the first appareance of fresh-water bivalves with *Archanodon*. In the marine environments a new extension of dysaerobic facies induced the development of small praecardioids (*Buchiola*) and pterioids (*Guerichia* = *Posidonia* in previous papers like Babin 1966) which were probably attached to floating algae or wood fragments (Fig. 9) although Grimm (1998) argues that *Buchiola* was infaunal. After the drastic decrease by the end of the Frasnian some bivalve faunas of the latest Famennian (Strunian) dominated by pteriomorphs and located at the southern margin of Laurussia initiated a new diversification (Amler 1996). Nevertheless, a clear decline in bivalve diversity is related to the global crisis near the end of the Devonian.

CONCLUSION

After a very discrete appearance during the Lower Cambrian and an obscure history during the Cambrian and the earlier Ordovician (Tremadoc), bivalves showed during the Arenig a dramatic phase of radiation corresponding to the famous Ordovician diversification. It seems that in a few millions years all or nearly all, the subclasses appeared. This drastic paleodiversification coincided with key innovations like the filibranchiate gills and the retention of the postlarval byssus in the adult. It was located on shallow clastic sea-beds, apparently on the peri-Gondwanan and Avalonian shelves. After a major dispersal during the Upper Ordovician corresponding notably to the diversification of the semi-endobyssate and epibyssate forms, the bivalves underwent a first decline with the latest Ordovician mass extinction. New radiations occurred during the Middle and Upper Silurian especially with the development of the cryptodonts related to particular dysaerobic environments, and with an explosive evolution of the pteriomorphs. The Devonian opened another period of paleodiversification at family, genus and species levels but with a progressive acquisition of a more modern cachet. The acme of this diversification took place during the late Early Devonian (Emsian), and the paleogeographic changes led to the establishment of cosmopolitan faunas. During this Period the bivalves dispersed into various marine environments and for the first time into fresh-water. A second decline coincided with the Late Devonian mass extinction.

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Reconstruction of ancestral character states in neocoleoid cephalopods based on parsimony

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Abstract: The Neocoleoidea, sister group to the Belemnoidea, includes all living cephalopod species except nautilids, as well as their immediate ancestors. Several hypotheses have been published about the morphology of ancestral neocoleoids. Ancestral states are easily inferred from fossils for some characters, such as 10 arms and the presence of an ink sac in basal coleoids or the presence of fins in ancient octopods. Many inferences are less strongly supported, though, and open to debate. We examine this problem using three cladograms resulting from analyses of morphology and DNA sequences (both mitochondrial and nuclear) from samples representing the full diversity of extant coleoids. Character states at three ancestral nodes (neocoleoid, octopodiform, and decapodiform) are reconstructed for 51 morphological characters using cladistic parsimony. Strong or moderate agreement among the three trees was found for almost 3/4 of the character-at-node reconstructions. The level of agreement among the trees varied among nodes, with strongest agreement found at the ancestral octopodiform node. However, some of these reconstructions seem unlikely to be correct. Changes in subclade resolution can exert varying effects on inferences about basal nodes. Because several subclades within the neocoleoids are not yet adequately resolved, we cannot be very confident in reconstructions of ancestral character states based solely on parsimony and we propose a provisional suite of character-state reconstructions including other sources of inference in addition to parsimony.

Key Words: squid, octopod, cuttlefish, morphology, evolution, phylogeny, cladistic consensus

The reconstruction of ancestral character states is an important step in unraveling the pathways of evolution and the changes that led to the present diversification in cephalopods. Naef (1921-3) relied on inference based on his extensive knowledge of comparative anatomy, embryology and paleontology to reconstruct generalized types from which all major coleoid groups could be derived. While not intending these to represent ancestral forms, he often felt that they did so. With the advent of more rigorous means of reconstruction, Naef's types are no longer acceptable for determining primitive states even though many of his conclusions ultimately may be supported. Subsequent efforts (*e. g.*, Bandel and Leich, 1986; Haas, 1997) have been based largely on inferences from fossils and the literature on anatomy of living cephalopods. Coleoid fossils, although rare, have occasionally been of use in determining the presence of some anatomical structures, such as an ink sac and ten arms in early coleoids and fins in ancient octopods (Young *et al.*, 1998). However, the fossil record of coleoids is exceptionally poor (Foote and Sepkoski, 1999), limiting its usefulness for character-state reconstruction.

An alternative approach is to use cladistic parsimony to infer character states at ancestral nodes of a cladogram. Cladistic reconstruction is based on rules that define which character states are most likely to occur at a node (Cunningham *et al.*, 1998). However, even with carefully and accurately defined character states, the results are not definitive. Nevertheless, inferences can be robust (Shultz *et al.*, 1996). We present here the first attempt to reconstruct ancestral character states for neocoleoid cephalopods (defined by Young *et al.*, 1998 as the sister group to the belemnoids and containing all extant coleoids) using parsimony analyses of cladistic hypotheses of phylogeny based on both morphological and molecular characters. We previously analyzed 50 morphological characters in order to determine relationships among the major groups of extant coleoid cephalopods (Young and Vecchione, 1996). These characters, for the most part, are those for which we try to construct evolutionary histories, although several characters have been redefined or replaced. We use the cladistic relationships inferred from morphology by Young and Vecchione (1996) as well as molecular phylogenies based on mitochondrial (Carlini and Graves, 1999) and nuclear (Carlini, 1998) DNA sequences.

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MATERIALS AND METHODS

Morphology

The data matrix used for the reconstructions is modified from Young and Vecchione (1996) and that paper can be consulted for much of the material examined. We have revised a few character definitions for the present analysis and have added or replaced a few characters. The character descriptions and data matrix are presented in Appendices 1-2. The consensus cladogram that Young and Vecchione considered the best estimate of neocoleoid phylogeny based on morphology is reproduced in Fig. 1. For the present study, we added Belemnoidea, the sister group to the neocoleoids, with character states derived from literature on fossil belemnoids. As is discussed below, reconstruction by parsimony does not always infer reasonable character states at ancestral nodes. We therefore offer a set of opinions in Appendix 3 about ancestral characters states. These opinions summarize the discussion of individual characters in Appendix 1. The parsimony solution is accepted unless some other source of information, such as paleontology or ontogeny, indicates that the inference from parsimony is particularly questionable.

Molecular sequences

Taxonomic sampling and details of extraction, amplification, cloning, and sequencing methods for DNA are presented by Carlini (1998). Many alternative phylogenetic analyses, using different models for cladogenesis, substitution, weighting, and combinations of molecular data sets are presented there as well, along with cladogram parameters such as tree lengths, consistency indices, bootstrap values, etc. We have selected here what we feel are the most reasonable phylogenetic hypotheses for separate mitochondrial and nuclear DNA sequences. The cladograms for both molecular data sets are fully resolved and are the most parsimonious from unweighted parsimony analysis. For reconstruction analyses, the trees representing these hypotheses were "pruned" so that the nodal structure of the trees was retained but terminal taxa are those for which morphological character states were assessed. Although the resulting trees appear pectinate, many of the terminal branches actually represent clades on the original molecular trees. Tree statistics are presented in the captions to Figs. 2 and 3.

Mitochondrial.– The cytochrome c oxidase I (COI) cladogram (Fig. 2) is similar to that of Carlini and Graves

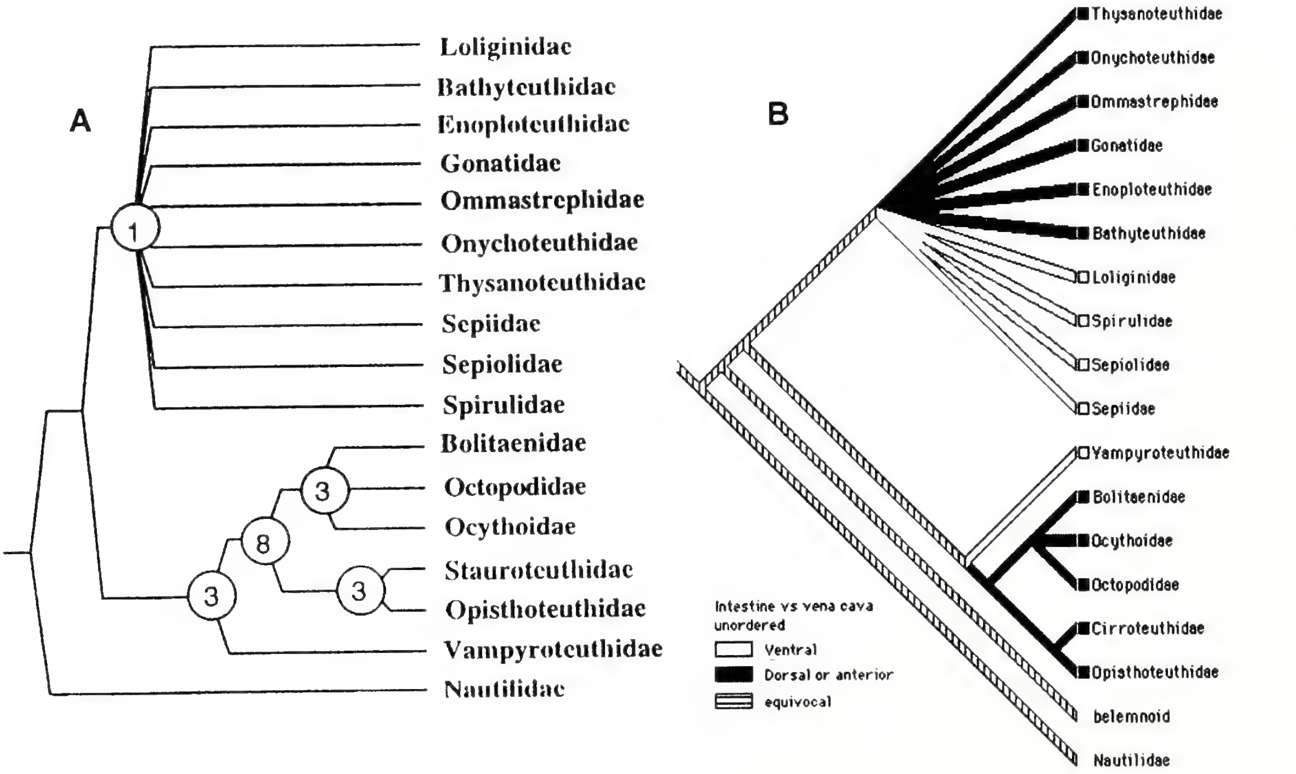
A
Loliginidae
Bathyteuthidae
Enoploteuthidae
Gonatidae
Ommastrephidae
Onychoteuthidae
Thysanoteuthidae
Sepiidae
Sepiolidae
Spirulidae
Bolitaenidae
Octopodidae
Ocythoidae
Stauroteuthidae
Opisthoteuthidae
Vampyroteuthidae
Nautilidae
1
3
8
3
3
B
Thysanoteuthidae
Onychoteuthidae
Ommastrephidae
Gonatidae
Enoploteuthidae
Bathyteuthidae
Loliginidae
Spirulidae
Sepiolidae
Sepiidae
Vampyroteuthidae
Bolitaenidae
Ocythoidae
Octopodidae
Cirroteuthidae
Opisthoteuthidae
belemnoid
Nautilidae
Intestine vs vena cava
unordered
Ventral
Dorsal or anterior
equivocal

Fig. 1. A. Morphological consensus cladogram based on Young and Vecchione (1996); consensus of 14 trees, tree length 46, consistency index 0.98, retention index 0.99. Numbers within circles are the number of unambiguous synapomorphies supporting the node at that location. **B.** Example of reconstruction cladogram based on A. In this and the following two figures, the same character (number 47, the position of the intestine relative to the vena cava) is used as an example of character-state reconstruction.

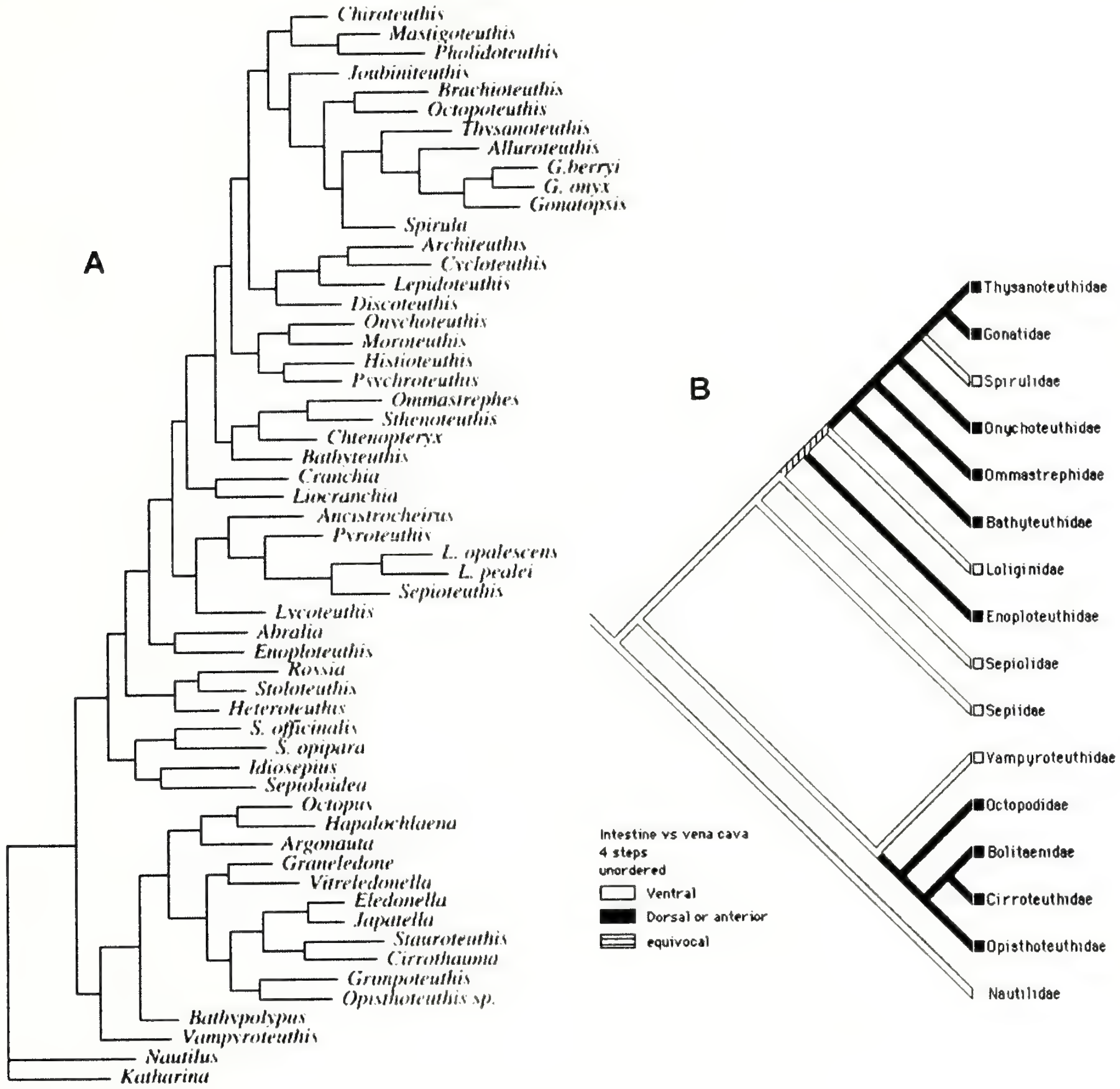
A
Chiroteuthis
Mastigoteuthis
Pholidoteuthis
Joubiniteuthis
Brachioteuthis
Octopoteuthis
Thysanoteuthis
Alluroteuthis
G. berryi
G. onyx
Gonatopsis
Spirula
Architeuthis
Cycloteuthis
Lepidoteuthis
Discoteuthis
Onychoteuthis
Moroteuthis
Histioteuthis
Psychroteuthis
Ommastrephes
Sthenoteuthis
Chtenopteryx
Bathyteuthis
Cranchia
Liocranchia
Ancistrocheirus
Pyroteuthis
L. opalescens
L. pealei
Sepioteuthis
Lycoteuthis
Abralia
Enoploteuthis
Rossia
Stoloteuthis
Heteroteuthis
S. officinalis
S. opipara
Idiosepius
Sepioloidea
Octopus
Hapalochlaena
Argonauta
Graneledone
Vitreledonella
Eledonella
Japetella
Stauroteuthis
Cirrothauma
Grimpoteuthis
Opisthoteuthis sp.
Bathypolypus
Vampyroteuthis
Nautilus
Katharina
B
Thysanoteuthidae
Gonatidae
Spirulidae
Onychoteuthidae
Ommastrephidae
Bathyteuthidae
Loliginidae
Enoploteuthidae
Sepiolidae
Sepiidae
Vampyroteuthidae
Octopodidae
Bolitaenidae
Cirroteuthidae
Opisthoteuthidae
Nautilidae
Intestine vs vena cava
4 steps
unordered
Ventral
Dorsal or anterior
equivocal

Fig. 2. A. Maximum parsimony cladogram based on DNA sequence of the mitochondrial gene for cytochrome *c* oxidase I; tree length 3763, consistency index 0.167, retention index 0.329. Branch lengths are proportional to changes in nucleotide sequence. **B.** Cladogram "pruned" for character-state reconstruction to retain nodal structure but reduce terminal taxa to those for which morphological data were assessed.

(1999), but the data were reanalyzed after including additional taxa. For the present paper, a 657 base-pair segment of the COI gene was analyzed for 55 species. Of these characters (base pairs), 297 were parsimony-informative. The result was a single most-parsimonious tree of length 3763 (Fig. 2).

Nuclear.– Preliminary phylogenetic analysis by Carlini (1998) on cephalopod actin sequences suggested that at least three paralogous actin genes had been amplified and cloned. These were subsequently discriminated among using restriction endonucleases. This finding was supported subsequently by genomic southern blotting, a more rigorous means of assessing gene copy number (Southern, 1975; Carlini *et al.*, 2000). Two of the three copies of the actin gene were analyzed in detail, and gene sequences from both paralogs were obtained from 26 taxa. The two genes, designated actin I and actin II, were concatenated in each taxon for a total of 1568 base pairs in the analysis of the combined data sets. The total number of parsimony-informative characters was 376. The cladogram presented here (Fig. 3) represents the single most parsimonious tree (length = 1581) derived from cladistic analysis of the combined actin genes.

Reconstructions

Ancestral character states have been reconstructed using the computer program MacClade (Maddison and Maddison, 1992), which finds the most parsimonious

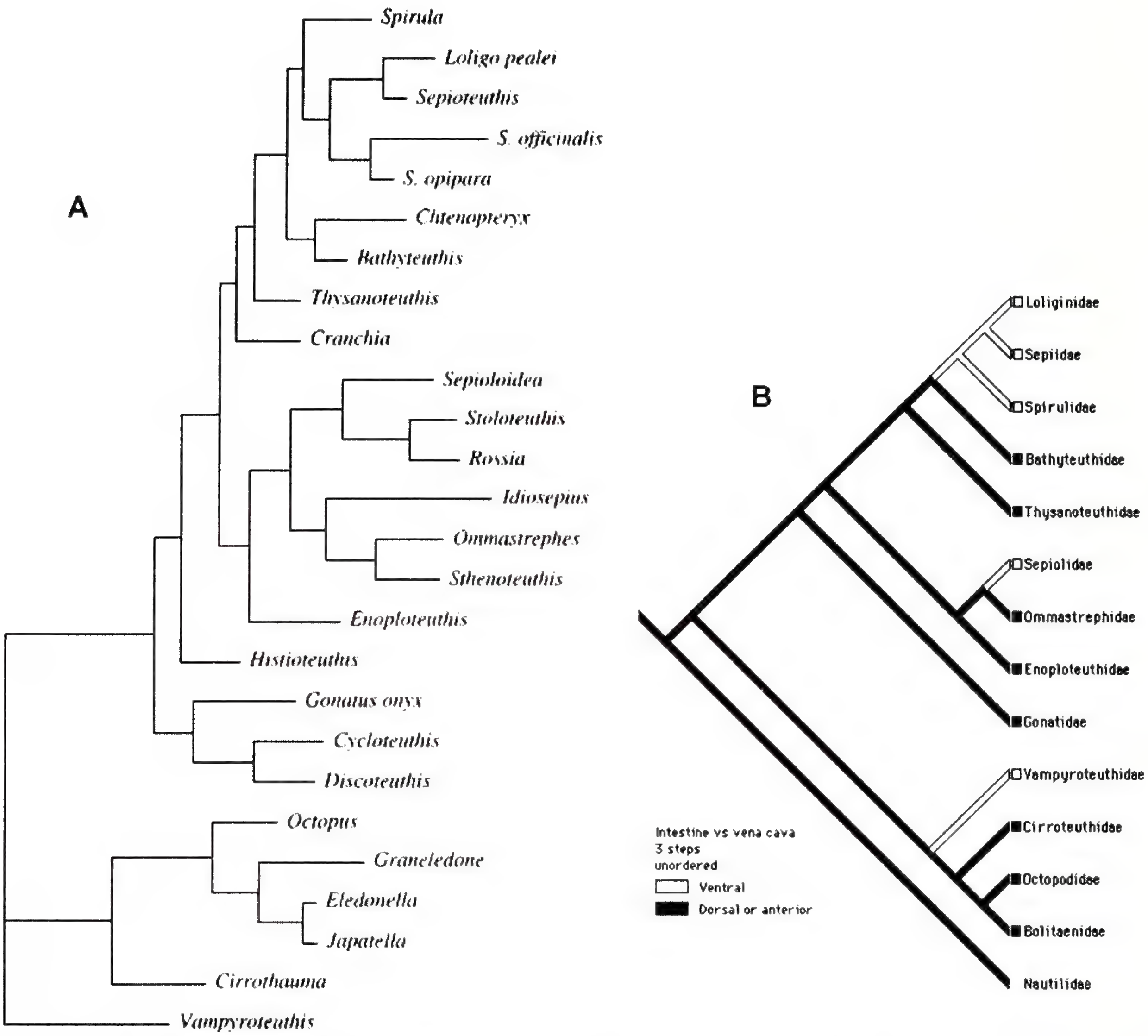
A
Spirula
Loligo pealei
Sepioteuthis
S. officinalis
S. opipara
Chtenopteryx
Bathyteuthis
Thysanoteuthis
Cranchia
Sepioloidea
Stoloteuthis
Rossia
Idiosepius
Ommastrephes
Sthenoteuthis
Enoploteuthis
Histioteuthis
Gonatus onyx
Cycloteuthis
Discoteuthis
Octopus
Graneledone
Eledonella
Japatella
Cirrothauma
Vampyroteuthis
B
Loliginidae
Sepiidae
Spirulidae
Bathyteuthidae
Thysanoteuthidae
Sepiolidae
Ommastrephidae
Enoploteuthidae
Gonatidae
Vampyroteuthidae
Cirroteuthidae
Octopodidae
Bolitaenidae
Nautilidae
Intestine vs vena cava
3 steps
unordered
Ventral
Dorsal or anterior

Fig. 3. A. Maximum parsimony cladogram based on combined DNA sequences of paralogs I and II for the nuclear gene for actin (Carlini, 1998; Carlini *et al.*, 2000); tree length 1581, consistency index 0.461, retention index 0.549. Branch lengths are proportional to changes in nucleotide sequence. **B.** Cladogram "pruned" for character-state reconstruction to retain nodal structure but reduce terminal taxa to those for which morphological data were assessed.

reconstructions. A characteristic of MacClade must be noted, however. In the case of polytomies, such as the decapod clade in the morphological tree, MacClade attempts to reconstruct character evolution as if the polytomy were resolved dichotomously by inventing an intercalated node between the polytomy and the basal dichotomous node outside of the polytomy. This convention is used when a character is polymorphic within a polytomy because the context of the basal node will vary with different resolutions of the phylogeny. However, for cases in which all taxa within a polytomy share a character state, we consider that state to be ancestral to the polytomy, rather than equivocal as would be reconstructed by MacClade. In the current presentation, this convention does not apply to the molecular trees, which are fully resolved, but affects many reconstructions based on the morphological tree, especially for the ancestral decapod node.

Character states were considered to be unordered except for three characters for analysis on the morphological tree. Young and Vecchione (1996) treated characters number 8 (arms II), 35 (superior buccal lobe) and 36 (subfrontal lobe) as ordered to infer the morphological phylogeny used here. Because these characters were considered to be ordered for development of the tree, we have treated them as ordered for character-state reconstruction based on the morphological tree. However, rather than impose these morphological assumptions on the independent molecular analyses, we considered all character states to be unordered for reconstructions based on gene trees.

To determine the degree of agreement among trees, we define a series of rules for the consensus column in Tables 1-3. (1) Strong agreement occurs when all three trees indicate the same character state at a node. (2) Moderate agreement is when two trees agree on a character state and the third reconstructs the character state as equivocal. (3) If two trees agree on a character state and the third reconstructs the character as an alternative state (as in majority-rules consensus) we consider this to be weak agreement. (4) When the reconstructed state is equivocal on two or three trees, this is listed as lack of consensus. (5) Disagreement occurs when two trees reconstruct a character as having different states and the third indicates that the state is equivocal.

RESULTS

Reconstructions of the states of 51 characters on morphological, nuclear (actins I and II), and mitochondrial (COI) trees are summarized in Table 1 for the ancestral neocoleoid node, Table 2 for the ancestral octopodiform node, and Table 3 for the ancestral decapod node. Of the total of 153 characters-at-nodes, strong agreement (all three trees agree on a character state) was found at 97, or 63.4%. If moderate agreement is included, the number increases to 112 (73.2%). Overall, the amount of agreement among trees, including strong, moderate, and weak agreement, was high at the decapodiform (43 characters) and octopodiform nodes (41 characters) and much lower (30 characters) at the neocoleoid node. The maximum strong agreement (38 characters) and least disagreement (1 character) among trees was found at the ancestral octopodiform node. Although the ancestral decapod node had a higher number of characters with strong agreement among trees (34) than did the ancestral neocoleoid node (25), the neocoleoid node had only two characters for which the trees disagreed; there were five such characters at the decapod node.

For some characters, agreement among trees was consistently strong at all focus nodes. The inferred character state remained unchanged among nodes for some of these characters. Examples include character 5, fins, character 10, suckers, and character 14, arm trabeculae, all present at all nodes. Other characters for which strong agreement was consistent within nodes exhibited changes in inferred state among nodes. Examples of these include character 7, the buccal crown (present in ancestral decapods but absent in octopodiforms), and character 19, the outer capsule of the statocyst (absent in ancestral neocoleoids and decapods but present in octopodiforms).

Several characters are otherwise noteworthy. Reconstructions of character 47, the position of the intestine relative to the vena cava, resulted in disagreement among trees at all three focus nodes (Figs. 1-3). Similarly, head width reconstruction, character 49, resulted in disagreement at two nodes and only weak agreement at the third. Assessment of states for both of these characters was particularly difficult (Appendix 1; Young and Vecchione, 1996).

Character 1, the phragmocone, is an example of a problem that is perhaps more important. Parsimony reconstructed the phragmocone as either absent or equivocal at every focus node on every tree. As discussed below, the phragmocone almost certainly was present at least in ancestral neocoleoids and decapods and possibly was present also in ancestral Octopodiformes. Therefore, these reconstructions of this character are almost certainly either incorrect (absent in actins at these nodes) or uninformative (equivocal elsewhere). Similarly, the reconstruction of the digestive gland, character 44, as fused at all ancestral nodes can be questioned based on embryological evidence of a paired origin of this structure. Such problems raise questions of reliability of the other reconstructions. We therefore present in Appendix 3 a summary of hypothesized ancestral character states based on ontogenetic and paleontological evidence in addition to parsimony, as discussed in Appendix 1.

DISCUSSION

Some neocoleoid morphological character states can be polarized by the extant outgroup, *Nautilus*, fossil cephalopods, or cephalopod embryology, but many cannot be polarized due to the lack of a living, closely-related outgroup for neocoleoids. Sufficient information exists, however, to make a first attempt at defining some of the basic character states (Appendix 1). The morphological hypothesis of relationships used here suffers from a lack of resolution within decapod and incirrate octopod clades. As a result, inferences are equivocal for character-state changes of many characters. The molecular analyses do not have this problem but suffer from unsubstantiated (*i. e.*, lack of bootstrap support) subclade relationships. Whereas the tree derived from the actin genes has the same major nodes as the morphological tree, the COI gene tree disagrees in the relationships within the Octopoda. Both molecular trees, however, are completely resolved within the Decapodiformes, although showing considerable disagreement between them, while the morphological tree depicts the Decapodiformes as an unresolved (*i. e.*, soft) polytomy. Although our analyses involve three quite different trees,

Table 1. Character states inferred using parsimony for the ancestral neocoleoid nodes of trees based on morphology, DNA sequence for combined actins I and II (of three paralogs), and DNA sequence for cytochrome oxidase subunit I. "Consensus" indicates agreement among trees with: --, indicating disagreement; -, indicating no consensus; a character state without an asterix indicating weak agreement; *, indicating moderate agreement; and **, indicating strong agreement.

Character		Morphology	Actins	COI	consensus
1	Phragmocone	Equivocal	Absent	Equivocal	-
2	Proostracum	Present	Present	Equivocal	*
3	Median Field	Equivocal	Equivocal	Equivocal	-
4	Shell Number	1	1	1	**
5	Fins	Present	Present	Present	**
6	Fin Cartilage	Equivocal	Equivocal	Equivocal	-
7	Buccal Crown	Equivocal	Equivocal	Equivocal	-
8	Arms II	Unmodified	Equivocal	Equivocal	-
9	Arms IV	Unmodified	Equivocal	Equivocal	-
10	Suckers	Present	Present	Present	**
11	Sucker Stalks	Equivocal	Equivocal	Equivocal	-
12	Sucker symmetry	Equivocal	Equivocal	Equivocal	-
13	Sucker Rings	Equivocal	Equivocal	Equivocal	-
14	Arm Trabeculae	Present	Present	Present	**
15	Arm Protective Membrane	Equivocal	Present	Present	*
16	Arm III Sucker Series	Equivocal	Equivocal	Equivocal	-
17	Arm I Web	Present	Equivocal	Present	*
18	Arm V Web	Equivocal	Equivocal	Equivocal	-
19	Statocyst Outer Capsule	Absent	Absent	Absent	**
20	Nephridial Coelom	2	2	2	**
21	Visceropericardial Coelom	Extensive	Extensive	Extensive	**
22	Dorsal Mantle Cavity	Absent	Absent	Absent	**
23	Nidamental Glands	Equivocal	Present	Present	*
24	Crop	Present	Present	Present	**
25	Branchial Canal	Equivocal	Present	Present	*
26	Mantle Septum	Equivocal	Equivocal	Equivocal	-
27	Mantle Adductor	Present	Equivocal	Equivocal	-
28	Funnel Valve	Present	Present	Present	**
29	Nuchal Cartilage	Present	Present	Present	**
30	Cornea	Absent	Absent	Absent	**
31	Right Oviduct	Present	Present	Present	**
32	Oviducal Gland Symmetry	Equivocal	Equivocal	Equivocal	-
33	Oviducal Gland Position	Terminal	Terminal	Terminal	**
34	Photosensitive Vesicle	Equivocal	Equivocal	Equivocal	-
35	Superior Buccal Lobe	Broad Sep.	Broad Sep.	Broad Sep.	**
36	Subfrontal Lobes	Equivocal	Equivocal	Equivocal	-
37	Arm-Mantle Muscle	Absent	Absent	Absent	**
38	Horizontal Arm Septae	Absent	Absent	Absent	**
39	Arm IV (III) Hectocotylus	Absent	Absent	Absent	**
40	Arm V Hectocotylus	Absent	Equivocal	Equivocal	-
41	Spermatophores	Present	Present	Present	**
42	DGDA, Number	Equivocal	Equivocal	Equivocal	-
43	DGDA, Location	Equivocal	Equivocal	Equivocal	-
44	Digestive Gland	Fused	Fused	Fused	**
45	Gonad: Coelom Coverage	Mostly Cov.	Mostly Cov.	Mostly Cov.	**
46	Posterior Salivary Gland	Post.to	Post.to	Post.to	**
47	Intestine vs Vena Cava	Equivocal	Dorsal	Ventral	--
48	Gill Filaments	Both Free	Both Free	Both Free	**
49	Head Width	1.00-1.49	Equivocal	0.50-0.99	--
50	Long. Mantle Muscle	Present	Present	Present	**
51	Arm Orientation	Anterior	Anterior	Anterior	**

Table 2. Character state inferences as in Table 1, but for the ancestral Octopodiformes nodes.

Character		Morphology	Actins	COI	consensus
1	Phragmocone	Absent	Absent	Absent	**
2	Proostracum	Present	Present	Equivocal	*
3	Median Field	Equivocal	Equivocal	Equivocal	-
4	Shell Number	1	1	1	**
5	Fins	Present	Present	Present	**
6	Fin Cartilage	Base + Core	Base + Core	Base + Core	**
7	Buccal Crown	Absent	Absent	Absent	**
8	Arms II	Modified	Equivocal	Equivocal	-
9	Arms IV	Unmodified	Unmodified	Unmodified	**
10	Suckers	Present	Present	Present	**
11	Sucker Stalks	Equivocal	Equivocal	Equivocal	-
12	Sucker symmetry	Radial	Radial	Radial	**
13	Sucker Rings	Equivocal	Equivocal	Equivocal	-
14	Arm Trabeculae	Present	Present	Present	**
15	Arm Prot. Membrane	Equivocal	Present	Present	*
16	Arm III Sucker Series	1	1	1	**
17	Arm I Web	Present	Present	Present	**
18	Arm V Web	Present	Present	Present	**
19	Statocyst Outer Capsule	Present	Present	Present	**
20	Nephridial Coelom	2	2	2	**
21	Visceropericardial Coelom	Extensive	Extensive	Extensive	**
22	Dorsal Mantle Cavity	Absent	Absent	Absent	**
23	Nidamental Glands	Absent	Absent	Absent	**
24	Crop	Present	Present	Present	**
25	Branchial Canal	Present	Present	Present	**
26	Mantle Septum	Equivocal	Equivocal	Equivocal	-
27	Mantle Adductor	Present	Equivocal	Equivocal	-
28	Funnel Valve	Present	Present	Present	**
29	Nuchal Cartilage	Present	Present	Present	**
30	Cornea	Absent	Absent	Absent	**
31	Right Oviduct	Present	Present	Present	**
32	Oviducal Gland Symmetry	Radial	Radial	Radial	**
33	Oviducal Gland Position	Terminal	Terminal	Terminal	**
34	Photosensitive Vesicle	Equivocal	Equivocal	Equivocal	-
35	Superior Buccal Lobe	Adjacent	Equivocal	Equivocal	-
36	Subfrontal Lobes	Incipient	Equivocal	Equivocal	-
37	Arm-Mantle Muscle	Absent	Absent	Absent	**
38	Horizontal Arm Septae	Absent	Absent	Absent	**
39	Arm IV (III) Hectocotylus	Absent	Absent	Absent	**
40	Arm V Hectocotylus	Absent	Absent	Absent	**
41	Spermatophores	Present	Present	Present	**
42	DGDA, Number	Single	Single	Single	**
43	DGDA, Location	Not in coel	Not in coel	Not in coel	**
44	Digestive Gland	Fused	Fused	Fused	**
45	Gonad: Coel. Coverage	Mostly Cov.	Mostly Cov.	Mostly Cov.	**
46	Posterior Salivary Gland	Post.to	Post.to	Post.to	**
47	Intestine vs Vena Cava	Equivocal	Dorsal	Ventral	--
48	Gill Filaments	Both Free	Both Free	Both Free	**
49	Head Width	1.00-1.49	0.50-0.99	0.50-0.99	
50	Long. Mantle Muscle	Present	Present	Present	**
51	Arm Orientation	Lateral	Lateral	Lateral	**

agreement among them in reconstructing specific character states provides some confidence in the reconstruction.

The Octopodiformes clade, which is most consistently resolved among the three trees, resulted in the most consistent reconstructions. The decapod clade, which was totally unresolved in the morphological tree considered here and was inconsistently resolved between the two molecular trees, resulted in many disagreements in reconstructed character states. The deeper node for ancestral neocoleoids was most noteworthy because of the large number

Table 3. Character state inferences as in Table 1, but for the ancestral Decapodiformes nodes.

Character		Morphology	Actins	COI	consensus
1	Phragmocone	Equivocal	Absent	Equivocal	-
2	Proostracum	Present	Present	Equivocal	*
3	Median Field	Equivocal	Narrow	Equivocal	-
4	Shell Number	1	1	1	**
5	Fins	Present	Present	Present	**
6	Fin Cartilage	Base Only	Base Only	Base Only	**
7	Buccal Crown	Present	Present	Present	**
8	Arms II	Unmodified	Unmodified	Unmodified	**
9	Arms IV	Tentacles	Tentacles	Tentacles	**
10	Suckers	Present	Present	Present	**
11	Sucker Stalks	Base & Neck	Base & Neck	Base & Neck	**
12	Sucker symmetry	Bilateral	Bilateral	Bilateral	**
13	Sucker Rings	Horny	Horny	Horny	**
14	Arm Trabeculae	Present	Present	Present	**
15	Arm Protective Membrane	Equivocal	Present	Present	*
16	Arm III Sucker Series	Equivocal	Equivocal	Equivocal	-
17	Arm I Web	Present	Absent	Present	
18	Arm V Web	Absent	Absent	Absent	**
19	Statocyst Outer Capsule	Absent	Absent	Absent	**
20	Nephridial Coelom	1	1	1	**
21	Visceropericardial Coelom	Extensive	Extensive	Extensive	**
22	Dorsal Mantle Cavity	Absent	Absent	Absent	**
23	Nidamental Glands	Equivocal	Present	Present	*
24	Crop	Absent	Absent	Absent	**
25	Branchial Canal	Equivocal	Present	Absent	--
26	Mantle Septum	Equivocal	Continuous	Continuous	*
27	Mantle Adductor	Present	Absent	Equivocal	--
28	Funnel Valve	Present	Present	Present	**
29	Nuchal Cartilage	Present	Present	Present	**
30	Cornea	Absent	Absent	Equivocal	*
31	Right Oviduct	Present	Present	Equivocal	*
32	Oviducal Gland Symmetry	Bilateral	Bilateral	Bilateral	**
33	Oviducal Gland Position	Terminal	Terminal	Terminal	**
34	Photosensitive Vesicle	Equivocal	In Cart.	In Cart.	*
35	Superior Buccal Lobe	Br.Separate	Br.Separate	Br.Separate	**
36	Subfrontal Lobes	Equivocal	Absent	Absent	*
37	Arm-Mantle Muscle	Absent	Absent	Absent	**
38	Horizontal Arm Septae	Absent	Absent	Absent	**
39	Arm IV (III) Hectocotylus	Absent	Absent	Absent	**
40	Arm V Hectocotylus	Absent	Present	Equivocal	--
41	Spermatophores	Present	Present	Present	**
42	DGDA, Number	Paired	Paired	Paired	**
43	DGDA, Location	Nephrocoel	Nephrocoel	Nephrocoel	**
44	Digestive Gland	Fused	Fused	Fused	**
45	Gonad: Coelomic Coverage	Covered	Covered	Covered	**
46	Posterior Salivary Gland	Post.to	Post. to	Post. to	**
47	Intestine vs Vena Cava	Equivocal	Dorsal	Ventral	--
48	Gill Filaments	Both Free	Both Free	Both Free	**
49	Head Width	1.00-1.49	0.00-0.49	Equivocal	--
50	Long. Mantle Muscle	Present	Present	Present	**
51	Arm Orientation	Anterior	Anterior	Anterior	**

of characters (19) for which there was no consensus among reconstructions, a result of the many characters for which reconstructions on individual trees were equivocal.

Reconstruction of ancestral character states using parsimony is a three-step process, including down-tree optimization, up-tree optimization, and final optimization reconciling the previous two steps (see Box 1 in Cunningham *et al.*, 1998 for a more complete explanation). As a result, changes in resolution of a subclade can have repercussions at nodes much deeper in a cladogram. For example, if the traditional, but controversial, suborder Sepioidea (= Sepiidae + Sepiadariidae + Sepiolidae + Idiosepiidae + Spirulidae) is considered to be a subclade within the Decapodiformes on the morphological cladogram used here, change in character states inferred at the ancestral decapod and neocoleoid nodes may (*e. g.*, character 3, median field of the proostracum, changes from absent to equivocal) or may not (*e. g.*, character 2, presence of the proostracum, is unchanged) occur, depending on the distribution of character states at the terminal branches. Parsimony-based reconstruction is also sensitive to assumptions about rates of evolution and probabilities of gain and losses (Cunningham *et al.*, 1998), questions about which very little information exists for neocoleoid cephalopods.

It is encouraging that some of these reconstructions are consistent with other sources of information. For instance, the reconstructed presence of fins, suckers, and arm trabeculae agree with inferences from the fossil record (Bandel and Leich, 1986). However, inability of parsimony to reconstruct the phragmocone as present based on the current phylogenetic hypotheses is troubling. A phragmocone is present in cuttlefish and *Spirula*, as well as in the extant outgroup, *Nautilus*. Additionally, phragmocones are known from fossil coleoids, including the belemnoid outgroup and early spirulids. It seems unlikely that such a complex structure evolved independently in all of these groups. Therefore, ancestral decapods and neocoleoids almost certainly possessed a phragmocone. *Vampyroteuthis* also has a structure of unknown function that could be a remnant of the siphuncle from a phragmocone (Young and Vecchione, 1996), indicating the possibility that ancestral octopodiforms may also have retained a phragmocone. The reconstructed state of this character as either absent or equivocal, together with the reconstructed state of the digestive gland, which contradicts embryological evidence (Appendix 1), greatly reduced our confidence in parsimonious reconstructions of ancestral character states based on our current knowledge of cephalopod phylogeny.

Character reconstruction requires known phylogenetic relationships. The analyses presented here, however, involve three trees depicting somewhat different phylogenetic relationships. The reconstruction of morphology in Appendix 3 is based on the assumption that the following relationships are correct: (1) The Belemnoidea and Neocoleoidea are sister groups (Young *et al.*, 1998). The apomorphic character states of the Neocoleoidea are: A. presence of suckers; B. absence of a nacreous layer in the shell; C. presence of fins. This foundation, while presently convincing, requires confirmation. (2) The Octopodiformes and Decapodiformes are monophyletic sister groups. Monophyly of the decapods was supported by a morphological cladistic study (Young and Vecchione, 1996) and molecular studies (Bonnaud *et al.*, 1997; Carlini, 1998; Carlini and Graves, 1999). Morphological support for monophyly was weak. The sole unambiguous morphological character found to unite the decapods was the modification of the fourth pair of arms into tentacles (belemnoids such as *Jeletzkya* and *Belemnotheutis* had 10 equal arms, presumably the primitive condition) although they share a variety of characters that could not be polarized. The monophyly of the Octopodiformes (Octopoda + Vampyromorpha; see Young *et al.*, 1998 for discussion of the proper name of this clade) was supported morphologically by: A. the shared outer capsule of the statocyst; B. modification of the second pair of arms; C. the position of the superior buccal lobe of the brain. Extant octopods have lost one pair of arms but the lost pair, apparently, is not the tentacles (arms IV), but rather arms II, which became retractile filaments in the Vampyromorpha; this problem is discussed in more detail by Young and Vecchione (1996; 1999) and Vecchione *et al.* (1999). (3) The Vampyromorpha and Octopoda are sister groups within the Octopodiformes. This genealogy has now been confirmed by separate cladistic studies of morphological and molecular data (Young and Vecchione, 1996; Bonnaud *et al.*, 1997; Carlini and Graves, 1999). (4) The Cirrata and Incirrata are sister groups within the Octopoda. This relationship has been supported by morphology (Young and Vecchione, 1996; Voight, 1997). A sister-group relationship betweeen the cirrates and incirrates is not supported by COI data (Carlini and Graves, 1999). This relationship was not adequately tested by the actin data in Carlini (1998) because few cirrates were included in the analysis due to difficulties in cloning cirrate actin DNA. However, the few cirrate taxa sampled for actin genes suggest a sister-group relationship between cirrates and incirrates. Furthermore, monophyly of the Octopoda is supported by both actin and COI.

Although reconstruction of ancestral character states is, of necessity, speculative (Frumhoff and Reeve, 1994), Shultz *et al.* (1996) concluded that such inferences can be remarkably robust. We have only begun the process of reconstruction here. Our understanding of coleoid evolution needs: (1) addition of characters to the list presented here, (2) resolution of the phylogenetic relationships among the decapods, the cirrates, and the incirrates and (3) greater knowledge of the developmental history of these characters in the embryos of all families considered. The latter will greatly increase our ability to define, assess, and polarize characters and clarify reconstructions that are presently ambiguous.

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APPENDIX 1. Comments on individual characters.

1. Phragmocone. States: 0- Present; 1- Absent. Young and Vecchione (1996) considered only the character "siphuncle." We expand the definition here to "phragmocone," which includes a siphuncle. The presence of a calcareous phragmocone in belemnoid fossils and *Nautilus* indicates that this structure was present in the ancestral coleoid. Among Neocoleoidea only sepiids and *Spirula* have a phragmocone. Since the highly complex phragmocone is unlikely to have arisen anew in neocoleoid evolution, we consider this an irreversible character. Pickford (1940) and Young and Vecchione (1996) have described what could be a remnant of a siphuncle in *Vampyroteuthis*. If correct, this supports the presence of a phragmocone in the early octopodiform. This possibility, however, is uncertain so the reconstruction of the early octopodiform, for now, must be "absent."

2. Proostracum. States: 0- Present; 1- Absent; 2- Entire. The coleoid proostracum (*i. e.*, the dorsal remnant of the living chamber of the pre-coleoid) is considered to be homologous with the gladius, except conus, in decapods and *Vampyroteuthis*. A proostracum does not exist in sepiids or *Spirula*. Some sepiolids (*e. g.*, *Rossia*) have a small anterior gladius that appears to represent the anterior end of the proostracum based on its embryonic development (Naef, 1921-1923). Other sepiolids and sepiadariids that lack a gladius obviously lack a proostracum. In octopods the internal shell, the apparent homologue of the gladius, is transversely elongate; it is single in cirrates and divided, when present, in incirrates. The structure of the octopod shell, however, cannot be related to the subdivisions seen in the gladius and the state of the proostracum in octopods coded as a "?". A proostracum is present in belemnoids. The complete living chamber of *Nautilus* is given the state "Entire." A narrow proostracum is present in the fossil sepioid *Groenlandibelus* and, therefore, absence in *Sepia* and *Spirula* can be interpreted as a loss.

3. Median field width of proostracum. States: 0- Broad; 1- Narrow; 2- Unrecognizable; 3- Absent; 4- Entire (*Nautilus*). In many extant coleoids, the gladius has a three-part structure: a medial field (= rhachis), lateral fields (= wings) and conus fields (these often form part of the terminal primary conus but may extend well anterior of the conus proper). A primary conus can be present or absent. The primary conus and conus fields are presumably remnants of the phragmocone while the rest of the gladius represents the proostracum. The anterior portion of the gladius of extant coleoids consists solely of the median field, which can be narrow (teuthoids and some sepioids) or broad in *Vampyroteuthis*. Belemnoids also have a broad proostracum, but its relationship to the median field is also uncertain so this has been coded as "?".

4. Shell number. States: 0- None; 1- One; 2- Two. The shell can be single (*i. e.*, teuthoid gladius) or double (incirrate shell) or absent (some sepiolids, incirrates). The double break in the mantle musculature in the position of stylets in the two incirrate families that lack stylets clearly indicates that the double state is ancestral to these families. The single condition as in cirrates clearly is the ancestral state as the double state in incirrates is derived from a single shell sac during embryology (Naef, 1921-1923). This interpretation contrasts with that of Voight (1997) that the double shell was ancestral to the Octopoda. We therefore consider this an ordered character and, as such, reconstruct the single condition as the ancestral incirrate condition.

5. Fins. States: 0- Present; 1- Absent. Fins are unknown in belemnoids (Young *et al.*, 1988), but are found throughout the decapods, as well as in *Vampyroteuthis* and the cirrate octopods, but are absent in incirrates. *Vampyroteuthis* has two pairs of fins. In the youngest specimens a juvenile fin is present. As the animal grows, a second pair of fins develops anteriorly, which persist into the adult stage; the juvenile fins are resorbed. Young and Vecchione (1996) presented evidence that the juvenile fin is the homologue of other cephalopod fins. MacClade reconstructs the ancestral incirrate condition as equivocal. However, fin folds are present in incirrate embryology (Naef, 1921-1923) suggesting that early incirrates had fins. In addition, fins are present in the fossil *Palaeoctopus newboldi*, which has been interpreted as an incirrate (Engeser and Bandel, 1988; Young *et al.*, 1998).

6. Fin cartilage. States: 0- Base only; 1- Base and core; 2- Absent. Both *Vampyroteuthis* (juvenile fin) and the cirrates have an extensive core of flexible cartilage extending through half or more of the fin (Young and Vecchione, 1996). This is absent from decapods, which have a cartilage at the base of each fin that doesn't penetrate the fin core. Incirrates lack fins and are coded as "?". Because the Decapodiformes are considered an unresolved bush in the morphological consensus tree, MacClade cannot determine whether the uniform decapod condition ocurred at the node of the bush or earlier.

7. Buccal crown. States: 0- Oral arms; 1- Present; 2- Absent. The buccal crown is present in decapods and absent in octopodiforms. It is thought to be homologous with the oral arms of *Nautilus* (Young and Vecchione, 1996). We therefore consider the states to be ordered: oral arms - buccal crown - absent. With this constraint, the ancestral neocoleoid condition is reconstructed as "present." The state in belemnoids is unknown. However, since the arm crown of belemnoids is known and oral arms are not present the options are either state 1 or 2.

8. Arms II. States: 0- Unmodified; 1- Modified; 2- Absent. Arms II are modified in *Vampyroteuthis* and lost in octopods. The likelihood that these assumptions are correct is discussed in Young and Vecchione (1996). On the assumption that modification preceeded loss, we order the states, which then reconstructs the ancestral octopodiform as "modified."

9. Arms IV. States: 0- Unmodified; 1- Modified (tentacles). Arms IV are modified as tentacles in decapods but are unmodified in octopodiforms. In several decapod species adults have only eight arms due to the loss of the tentacles during ontogeny. The eight-armed condition in these decapods is clearly secondary as tentacles are present in paralarvae.

10. Suckers. States: 0- Absent; 1- Present. *Nautilus* lacks suckers as, apparently, did the belemnoids. Donovan and Crane (1992) reported possible suckers in *Belemnotheutis*, but these are more likely the muscular bases of hooks (Young *et al.*, 1998). Because all living coleoids have suckers, we consider the presence of suckers to be a neocoleoid synapomorphy as suggested by Engeser and Bandel (1988) and as reconstructed by MacClade.

11. Sucker stalks. States: 0- Base and neck; 1- Base and plug; 2- Cylinder. The suckers of decapods have stalks that are cone-like and terminate in a constricted, narrow neck. Octopods have broad, cylindrical sucker stalks. *Vampyroteuthis* has stalks that are unique (state 1), but in some ways intermediate between the decapod and octopod conditions. Polarity among these character states is presently undetermined.

12. Sucker symmetry. States: 0- Radial; 1- Bilateral. Decapods are characterized by having bilaterally symmetrical suckers. Octopods and *Vampyroteuthis* have suckers that are radially symmetrical, and reconstruction of the ancestral octopodiform is therefore with radial suckers. Polarity among these character states is presently undetermined.

13. Sucker rings. States: 0- Cuticular; 1- Absent; 2- Horny. Octopods have cuticular sucker rings; decapods have horny sucker rings often modified into hooks, and *Vampyroteuthis* lacks sucker rings. Polarity among these character states is presently undertermined.

14. Arm trabeculae. States: 0- Present; 1- Absent. Trabeculae (including their apparent homologues, cirri) are present in many decapods, *Vampyroteuthis* and the cirrate octopods. Within decapods, however, trabeculae are often reduced or absent. Belemnoids and *Nautilus* were coded as "?"; belemnoids because of uncertainty and *Nautilus* because the arms are so different that the character is not applicable. Haas (1989) has proposed that belemnoid arm hooks could be homologous with

neocoleoid trabeculae.

15. Arm protective membranes. States: 0- Present; 1- Absent. Although protective membranes are present between distal trabeculae on the arms of *Vampyroteuthis*, they are completely absent from octopods and from one decapod family (Sepiolidae); all other decapods examined possess protective membranes although they are often reduced. We consider that the absence from the sepiolids is likely due to secondary loss (as reconstructed in the molecular data sets). Under this constraint, the ancestral decapod, octopodiform and neocoleoid are reconstructed as "present."

16. Armature series on arms III. States: 0- One; 1- Two; 2- Four; 3- > Four. In Octopodiformes the suckers are either one or two series, or occasionally a combination of these states. However, many incirrates with two series of arm suckers have a single series as hatchlings. This supports a reconstruction of a single series in the ancestral octopodiform. Recent decapods have their armature in two, four or sometimes more than four series. In decapods, as in octopods with two series, the suckers are staggered, suggesting a sequence in which single series become double series and double series become quadruple series (as noted by Naef, 1921-1923). Unfortunately there are no concrete data to support this hypothesis and the condition in the ancestral decapod must be reconstructed as equivocal. In *Vampyroteuthis* and the cirrates, there are two trabeculae/sucker on the sucker-bearing portion of the arms. In decapods where two sucker series exist, there is one trabecula/sucker; where four sucker series are present, there is one trabecula/two suckers. Trabeculae, therefore, appear to be progressively lost as the number of sucker series increases. That is, the trabecula is lost from the side of the sucker that no longer is adjacent to the margin of the arm. This is a scenario that would be expected as one series becomes multiple series.

17. Arm webs, dorsal. States: 0- Absent; 1- Present. Well-developed webs between the dorsal pair of arms are present in most octopodiforms and some decapods. We consider the dorsal sector to be representative of web development between the dorsal six arms.

18. Arm webs, ventral. States: 0- Absent; 1- Present. A web between the ventral arms is present in all major octopodiform lineages, but is uniformly absent in decapods.

19. Outer capsule of statocyst. States: 0- Absent; 1- Present. An outer capsule is present in the statocyst of *Vampyroteuthis* and all octopods. It is absent in decapods and *Nautilus*.

20. Nephridial coelom. States: 0- Two; 1- One. Octopodiforms and *Nautilus* have two nephridial coeloms (one pair), whereas one (fusion) coelom is uniform in decapods. This character was not used by Young and Vecchione (1996) to support decapod monophyly due to difficulties in determining if fusion occurred more than once.

21. Visceropericardial coelom. States: 0- Extensive; 1- Reduced. An extensive visceropericardial coelom is found in *Nautilus*, the decapods and *Vampyroteuthis*. Reduction in octopods is a synapomorphy in this clade.

22. Dorsal mantle cavity. States: 0- Absent; 1- Present. The dorsal mantle cavity as defined by Young and Vecchione (1996) is an autapomorphy in the Octopoda. This structure bears considerable resemblance to the large nuchal cavity of *Spirula* and we suspect that both were derived in the same manner. The nuchal cavity is a space where the dorsal mantle articulates with the head. In most decapods the gladius (= proostracum) extends to the anterior tip of the mantle where a cartilaginous reinforcement of the shell sac articulates with the nuchal cartilage. *Spirula* lacks a proostracum and the Recent octopod shell doesn't reach the anterior mantle margin, yet a proostracum was probably present in the ancestors of both groups (see character no. 2). Perhaps the proostracum was progressively reduced over evolutionary time, and as the proostracum receeded posteriorly, the nuchal cavity increased accordingly. The later anterior fusion of the head and mantle margin in the Octopoda formed the dorsal mantle cavity. This scenario is further supported by the reduced gladius found in the Idiosepiidae. Here the gladius is absent both anteriorly and posteriorly. Anteriorly, an expanded nuchal cavity is also present. The convergent condition in *Spirula* and *Idiosepius* occurred without the dorsal mantle and head fusing. For species in which the head-mantle fusion has occurred without a posterior regression of the gladius (*e.g., Sepiola, Vampyroteuthis*), the nuchal cavity has been obliterated.

23. Nidamental glands. States: 0- Absent; 1- Present. These glands produce some of the external coatings on eggs as they are spawned. The glands are found in *Nautilus* and nearly all decapods except the Enoploteuthidae. Because of their absence in the latter family, reconstructions of the ancestral neocoleoid and decapod based on morphology are equivocal. However, we consider that the absence in the Enoploteuthidae, which spawn individual eggs strung together in a single gelatinous strand rather than gelatinous or encapsulated egg masses, is probably a secondary loss (as predicted by the molecular trees). It is unlikely that nidamental glands evolved twice (*i. e.*, decapods and *Nautilus*). With this constraint we reconstruct the ancestral decapod as having nidamental glands.

24. Crop. States: 0- Present; 1- Absent. A crop, defined here as a swelling or diverticulum of the esophagus, is present in *Nautilus*, *Vampyroteuthis* and most octopods. The loss of the crop, therefore, is an apomorphy for the Decapodiformes

25. Branchial canal. States: 0- Absent; 1- Present; 2- Secondary loss. A branchial canal is present in teuthoids, *Vampyroteuthis* and in the incirrate octopods, but absent from *Nautilus*, some decapods (sepioids) and the cirrates. The cirrates, however, have highly modified gills, which likely resulted in the loss of the canal independent of the loss in some decapods; the cirrate condition, therefore, is coded as a different state. Because the condition in the Decapodiformes is polymorphic, the ancestral decapod state is equivocal.

26. Median mantle septum. States: 0- Absent; 1- Present and continuous; 2- Present but open posteriorly; 3- Present as a blood vessel only. The visceral mass of *Spirula* is highly distorted by the presence of a coiled phragmocone and the mantle septum is absent except for the presence of the median mantle artery. Because this artery normally passes along the anterior margin of the septum, we consider the artery to represent the mantle septum in *Spirula*. This makes the presence of the septum uniform within the decapods as it is in the octopods. It is absent in *Vampyroteuthis*. The septum is open in all octopods except the cirrate *Grimpoteuthis glacialis*.

27. Mantle adductor. States: 0- Absent; 1- Present. The mantle adductor is uniformly present in the octopods and in the sepiolid decapods. The sepiolids have a strong effect on the reconstructions because decapod relationships are unresolved. We consider that the mantle adductor in octopods and sepiolids is a result of convergence (as suggested by the molecular actin tree). Muscles are typically associated with the mantle septum and hypertrophy of these muscles into a mantle adductor in separate lineages could easily occur. Under this constraint, the ancestral decapod condition is reconstructed as "present."

28. Funnel valve. States: 0- Present; 1- Absent. The funnel valve is present in *Nautilus, Vampyroteuthis* and most decapods. Although it is absent among decapods in some cranchiids and *Planctoteuthis*, the presence of the funnel valve in some members of these families suggests that this is a secondary loss.

29. Nuchal cartilage. States: 0- Present; 1- Absent. Although *Nautilus* doesn't offer any information on this structure, the nuchal cartilage is present in *Vampyroteuthis* and nearly all decapods (it is absent in some sepiolids and sepiadariids; its presence in some members of these families indicates that these are secondary losses).

30. Cornea. States: 0- Absent; 1- One part; 2- Two part. A cornea is absent in the cirrate octopods and *Vampyroteuthis* as well as many decapods. A two-part cornea is present in the incirrates. A one-part cornea is present in some decapods. These two types of corneas are considered to be independent derivatives of the eyelid. We did not find any corneas in the cirrates examined. However, *Opisthoteuthis* possesses inner, muscular,

pigmented eyelids in the form of convex, overlapping horizontal membranes. These have the same form as the clear, fixed corneas of the incirrates and can be interpreted either as the forerunner or remnant of a cornea.

31. Right oviduct. States: 0- Absent; 1- Present. The right oviduct is present in many decapods (it is absent in loliginids and sepioids), *Vampyroteuthis*, incirrate octopods and *Nautilus*. This means that the oviducts were paired since the left oviduct is present in all neocoleoids. The absence of the right oviduct in cirrate octopods and some decapods represents convergence.

32. Oviducal gland symmetry. States: 0- Radial; 1- Bilateral; 2- Asymmetrical. The oviducal glands are radially symmetrical in the octopodiform lineage and bilaterally symmetrical in the decapod lineage but asymmetrical in *Nautilus*. The character therefore cannot be polarized.

33. Position of oviducal gland. States: 0- Terminal; 1- Subterminal. The oviducal gland is located in the terminal position in decapods, *Vampyroteuthis* and *Nautilus*, but is subterminal in the Octopoda.

34. Photosensitive vesicles. States: 0- Within cephalic cartilage; 1- Above funnel; 2- On stellate ganglia. In some decapods the photosensitive vesicles lie on the optic stalks of the brain, and in others they have moved off the stalks but lie mostly within the confines of the cephalic cartilage with nerves running to the optic stalks. The photosensitive vesicles lie on the stellate ganglia in octopods and their nerves pass through this ganglion and into the pallial nerve (J. Z. Young, 1977), which leads to the brain. In *Vampyroteuthis*, they lie just dorsal to the funnel and their nerves follow the posterior funnel nerve toward the brain. Thus, the three major lineages have vesicles in different localities. We suspect that nerves from photosensitive vesicles of all cephalopods enter the brain in the region of the optic stalk and that the vesicles originally evolved at this location. If so, the decapod state would be the plesiomorphic state for the Neocoleoidea. Unfortunately, this cannot be confirmed at present and this reconstruction is equivocal. The reconstruction further assumes that the unknown state in *Thysanoteuthis* will conform to that of other decapods.

35. Superior buccal lobes. States: 0- Broadly separated; 1- Adjacent; 2- Fused. The superior buccal lobes are far removed from the brain in *Nautilus* and decapods but are adjacent to the brain in *Vampyroteuthis* and are fused with the brain in octopods, with the greatest compaction occurring in the cirrate octopods. The situation in *Vampyroteuthis* is actually somewhat more intimate than "adjacent"; the lateral edges of the superior buccal lobe and posterior buccal lobes lie within the same connective tissue covering. The state of this character strongly reflects the distance between the brain and the buccal mass. Young and Vecchione (1996) considered this to be an ordered character: separate - adjacent - fused.

36. Inferior frontal system of the brain. States: 0- Absent; 1- Insipient; 2- Present. The inferior frontal system of incirrates deals with the use of chemotactile information from the arms (J. Z. Young, 1971). This system is composed of the posterior buccal, lateral inferior frontals, subfrontals and the median inferior frontal lobe (J. Z. Young, 1971). The system develops embryologically from the posterior buccal lobes (J. Z. Young, 1965) and is best developed in incirrate octopods, but is present in cirrates as well. In decapods only the posterior buccal lobes are present. In *Vampyroteuthis*, complexities of the posterior buccal lobes and their connections have been interpreted as an incipient inferior frontal system (J. Z. Young, 1977). We consider this to be an ordered character with the vampyromorph condition intermediate, as did Young and Vecchione (1996). The reconstruction further assumes that the unknown state in *Thysanoteuthis* will conform to that of other decapods.

37. Arm-mantle muscle. States: 0- Present; 1- Absent. Special muscle bundles run between the bases of the dorsal arms and the dorsal, anterior end of the mantle in the octopods. This feature is a synapomorphy of the octopods and defines the dorsal head-mantle fusion peculiar to them. These muscles are not present in the head-mantle fusions of *Vampyroteuthis* or some decapods.

38. Horizontal arm septa. States: 0- Absent; 1- Present. Peculiar orally concave horizontal septa extend along the arms of all cirrate octopods and are found nowhere else. The arms of the incirrate bolitaenids have a somewhat similar arrangement but with different septal attachments; this condition was considered as a separate character state by Young and Vecchione (1996). Concave horizontal septa, therefore, is an apomorphy for the Cirrata.

39. Arm IV hectocotylizaton. States: 0- Absent; 1- Present. Because of the loss of arm pair II in octopods (see character 8), arms IV are generally considered by students of neocoleoids to be the "third" pair of arms. Modification of one of the "third" arms (actually arms IV) for the transmission of spermatophores is a synapomorphy in the incirrate octopods.

40. Arm V hectocotylization. States: 0- Absent; 1- Present. Modification of one of the ventral arms (arms V are often referred to as arms IV, not counting the tentacles in decapods as an arm pair) for the transmission of spermatophores occurs among many decapods, but not all, and is absent in other lineages.

41. Spermatophores. States: 0- Present; 1- Sperm packets; 2- Encapsulated coil. Typical spermatophores with an ejaculatory apparatus are found throughout the coleoids with the exception of the cirrate octopods. The presence of special sperm packets in cirrates, apparently a secondary simplification, is an apomorphy in this group.

42. Digestive-gland-duct appendages (DGDA), number. States: 0- Single; 1- Paired. In nearly all decapods the DGDA are spread along the ducts between the digestive gland and the caecum. In *Vampyroteuthis* and the octopods they are fused and compacted against the digestive gland. In a few genera of decapods (*e. g., Batoteuthis*, various cranchiids) compaction exists but not fusion. Because the appendages are lacking in *Nautilus*, polarity is uncertain.

43. DGDA, location. States: 0- In nephridial coelom; 1- Not in nephridial coelom. The DGDA in the decapods are located within (actually surrounded by) the dorsal sac of the nephridial coelom but they are separate from this coelom in the octopodiform lineage. The states cannot be polarized.

44. Digestive gland. States: 0- Many; 1- Paired; 2- Fused. Digestive glands are paired only in *Sepia, Spirula* and *Sepiadarium*. *Nautilus* has numerous digestive glands. Parsimony indicates the single state to be ancestral. However, embryology clearly indicates the paired origin of this structure in some species having a single organ (*e.g., Loligo, Octopus*). We therefore consider that paired glands are the ancestral neocoleoid state in spite of their reconstruction as fused. If this is correct, secondary fusion to produce a single digestive gland has occurred in more than one lineage.

45. Gonad: coelomic covering. States: 0- Mostly covered; 1- Less than 50% covered. In most neocoleoids the gonad lies suspended in the visceropericardial coelom (virtually 100% covered) although lined by the coelomic epithelium. In incirrates much less, but in excess of 50%, is covered. A synapomorphic condition exists in the cirrates in which less than 50% of the gonad lies within the coelom.

46. Posterior salivary glands. States: 0- Absent; 1- Posterior to cephalic cartilage; 2- On or in buccal mass. The posterior salivary glands are usually found posterior to the brain and the cephalic cartilage. Only in the cirrate octopods are they found anterior to the brain and on or in the buccal mass. Therefore, this latter state is synapomorphic in cirrates.

47. Intestine: position relative to vena cava. States: 0- Ventral; 1- Dorsal/anterior. The intestine either runs dorsal/anterior to the vena cava (*Vampyroteuthis*, sepioids, loliginids) or ventral to it (oegopsids and octopods). This character exhibits homoplasy (Young and Vecchione, 1996) and outside the Octopoda ancestral states are equivocal.

48. Gill filaments. States: 0- Both free; 1- Outer attached; 2-

Both attached. The tips of the gill filaments are free in some taxa (*Nautilus*, many decapods, *Vampyroteuthis*, some cirrates). Alternatively, one (Onychoteuthididae, Ocythoidae) or both (many incirrates, Opisthoteuthididae) filaments may be attached to the gill base.

49. Head width proportional to eye diameter. States: 0- 0-0.49; 1- 0.5-0.99; 2- 1.0-1.49; 3- 1.5-1.99; 4- 2.0-2.49; 5- 2.5-2.99; 6-3.0-3.49; 6- 3.5-3.99; 7- 4.0-4.49; 8- 4.5-4.99. Young and Vecchione (1996) attempted to quantify the head width by using the eye diameter as a size standard against which to measure head width. They compared the eye diameter to the head width measured between the extremities of the lenses and expressed it as a ratio. The method was only partially satisfactory because animals with dorsally tilted eyes added a complication and, in some, the eye size is simply a poor size standard for judging head width. Because of these problems, results of reconstruction must be taken cautiously. A general pattern, nevertheless, exists with many of the oegopsids (and *Spirula*) having narrow heads, most sepioids, loliginids and *Vampyroteuthis* having intermediate head widths and octopods having broad heads. The head of *Vampyroteuthis* is actually rather broad but since the eyes are especially large in this species our measure doesn't reflect head size very well. Head width, in addition, seems to be a good, but not absolute, indicator of body width.

50. Longitudinal mantle muscles. States: 0- Present; 1- Absent. Mantles of many decapods are composed mostly of circular and radial muscles but thin, discontinuous layers of longitudinal muscles are also present on the outer surface of the mantle especially near the anterior and posterior ends of the mantle. All groups examined, with the exception of a few families of decapods, had longitudinal muscles.

51. Arm orientation. States: 0- Lateral; 1- Anterior. In the relaxed position, the arms of some cephalopods extend laterally away from the head while in others they extend anteriorly. The arms of all cephalopods, however, are very muscular and capable of moving through a wide range in orientation. We have searched for anatomical correlates (*e. g.*, how the arms relate to the buccal mass) of the two basic orientations in order to quantify the character states. We have, unfortunately, been unsuccessful; as a result this character has not been adequately surveyed. Nevertheless, there seems to be little question that a basic difference in arm orientation exists between the octopodiform lineage and the decapods. When the arms of the former (typically oriented laterally) bend forward, their base near the buccal mass generally extends first laterally then anteriorly as the arm curves forward. In contrast, one usually finds that, in decapods, arms are typically directed forward and when they are directed laterally the orientation at the base generally extends first anteriorly then laterally as the arm curves aborally. The lateral orientation is most obvious in *Vampyroteuthis* and the cirrate octopods. The difference is less obvious in some of the muscular pelagic octopods such as *Ocythoe*. *Nautilus* tentacles and the preserved arms of some belemnoids (*e. g., Belemnotheutis*) show anteriorly oriented arms.

APPENDIX 2. Matrix of morphological character states used for reconstructions. Explanation of numerical designations for character states is presented in Appendix 1. Most of the material examined is listed in Young and Vecchione (1996).

	0				1				2							3 4			5
Character	1	2	3	4	56789012345	6	7	89	0	123	4	5	6	78	9	012345678901234567	8	9	01
Bathyteuthidae	1	0	1	1	00101101210	2	1	00	1	001	1	1	1	00	0	011000010000102011	0	0	01
Enoploteuthidae	1	0	1	1	00101101200	1	0	00	1	000	1	1	1	00	0	011000010010102011	0	0	01
Gonatidae	1	0	1	1	00101101200	2	0	00	1	001	1	1	1	00	0	01100001001 1?02011	0	0	11
Loliginidae	1	0	1	1	00101101200	1	0	00	1	001	1	1	1	00	0	101000010010102010	0	(12)	01
Ommastrephidae	1	0	1	1	00101101200	1	0	00	1	001	1	1	1	00	0	011000010010102011	(01)	0	11
Onychoteuthidae	1	0	1	1	00101101210	1	0	00	1	001	1	1	1	00	0	011000010000102011	1	0	01
Sepiidae	0	1	3	1	00101101200	2	1	00	1	001	1	0	1	00	0	101000010010101010	0	2	01
Sepiolidae	1	(01)	(13)	(01)	00101101211	(12)	(01)	00	1	001	1	0	1	10	(01)	101000010000102010	0	1	01
Spirulidae	0	1	3	1	00101101210	2	1	00	1	001	1	0	3	00	0	001000010010101010	0	0	01
Thysanoteuthidae	1	0	1	1	00101101200	1	0	00	1	001	1	1	1	00	0	0110???10010102011	0	0	11
Bolitaenidae	1	1	?	0	1?220120011	0	1	11	0	110	0	1	2	11	1	21012220010?012011	2	3	00
Octopodidae	1	?	?	2	1?220120011	(01)	1	11	(01)	110	0	1	2	11	1	210122200100012011	2	5	00
Ocythoidae	1	1	?	0	1?220120011	1	0	01	0	110	0	1	2	11	1	210122200100012011	1	4	01
Cirroteuthidae	1	?	?	1	01220120001	0	1	11	0	110	(01)	2	2	11	1	000122201001012121	0	4	00
Opisthoteuthidae	1	?	?	1	01220120001	0	1	11	0	110	0	2	(12)	11	1	0001?2201001012121	2	?	?0
Vampyroteuthidae	1	0	0	1	01210110100	0	1	11	0	000	0	1	0	?0	0	010011110000012010	0	2	00
Nautilidae	0	2	4	1	1?0??0?????	?	?	?0	0	001	0	0	?	?0	?	0120?0?10??2??000?	0	9	01
belemnoid	0	0	?	1	1??000?????	?	?	??	?	???	?	?	?	??	?	??????????????????	?	?	?1

APPENDIX 3. Provisional reconstructions of character states for ancestral nodes based subjectively on evidence from ontogeny and paleontology as well as on morphological parsimony.

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ancestral coleoid	0	0	?	1	1	?	?	0	0	0	?	?	?	?	?	?	?	?	0	0
Belemnoid	0	0	?	1	1	?	?	0	0	0	?	?	?	?	?	?	?	?	0	0
Ancestral neocoleoid	0	0	?	1	0	?	1	0	0	1	?	?	?	0	0	?	1	?	0	0
Ancestral decapod	0	0	?	1	0	0	1	0	1	1	0	1	2	0	0	?	1	0	0	1
Decapodiformes	0/1	0/1	1/2	1	0	0	1	0	1	1	0	1	2	0/1	0/1	1/2	0/1	0	0	1
Ancestral octopodiform	1	0	?	1	0	1	2	1	0	1	?	0	?	0	0	0	1	1	1	0
Vampyromorpha	1	0	0	1	0	1	2	1	0	1	1	0	1	0	0	0	1	1	1	0
Ancestral octopod	1	?	?	1	0	1	2	2	0	1	2	0	0	0	1	0	1	1	1	0
Ancestral cirrate	1	?	?	1	0	1	2	2	0	1	2	0	0	0	1	0	1	1	1	0
Cirrata	1	?	?	1	0	1	2	2	0	1	2	0	0	0	1	0	1	1	1	0
Ancestral incirrate	1	?	?	1	0	1	2	2	0	1	2	0	0	1	1	0	1	1	1	0
Incirrata	1	1/?	?	0/2	1	?	2	2	0	1	2	0	0	1	1	0/1	0/1	0/1	1	0/1

Character number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Ancestral coleoid	0	0	1	0	?	?	?	0	?	0	1	?	0	?	0	?	1	0	?	?
Belemnoid	0	0	1	0	?	?	?	0	?	0	1	?	0	?	0	?	1	0	?	?
Ancestral neocoleoid	0	0	1	0	?	?	?	0	0	0	1	?	0	?	0	?	1	0	0	0
Ancestral decapod	0	0	1	1	?	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0
Decapodiformes	0	0	0/1	1	0/1	1/3	0/1	0	0/1	0/1	0/1	1	0	0	0	0	1	0	0	0/1
Ancestral octopodiform	0	0	0	0	1	?	?	0	0	0	1	0	0	?	1	1	1	0	0	0
Vampyromorpha	0	0	0	0	1	0	?	0	0	0	1	0	0	1	1	1	1	0	0	0
Ancestral octopod	1	1	0	0	1	1	1	1	1	0	1	0	1	2	2	2	0	0	0	0
Ancestral cirrate	1	1	0	0	2	1	1	1	1	0	0	0	1	2	2	2	0	1	0	0
Cirrata	1	1	0	0/1	2	1/2	1	1	1	0	0	0	1	2	2	2	0	1	0	0
Ancestral incirrate	1	1	0	0	1	1	1	1	1	2	1	0	1	2	2	2	0	0	1	0
Incirrata	1	1	0	0	1	1	1	1	1	2	1	0	1	2	2	2	0	0	1	0

Character number	41	42	43	44	45	46	47	48	49	50	51
Ancestral coleoid	?	?	?	?	0	?	?	0	?	0	1
Belemnoid	?	?	?	?	0	?	?	0	?	0	1
Ancestral neocoleoid	0	?	?	1	0	1	?	0	2	0	1
Ancestral decapod	0	1	0	1	0	1	?	0	2	0	1
Decapodiformes	0	?/1	0	1/2	0	1	0/1	0/1	0-2	0/1	1
Ancestral octopodiform	0	0	1	2	0	1	?	0	2	0	0
Vampyromorpha	0	0	1	2	0	1	0	0	2	0	0
Ancestral octopod	0	0	1	2	0	1	1	?	4	0	0
Ancestral cirrate	1	0	1	2	1	2	1	?	4	0	0
Cirrata	1	0	1	2	1	2	1	0/2	4	0	0
Ancestral incirrate	0	0	1	2	0	1	1	?	4	0	0
Incirrata	0	0	1	2	0	1	1	1/2	3-5	0	0/1

Concerning the concept of extinct classes of Mollusca: or what may/may not be a class of mollusks

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Abstract: In the notion of classes, all of whose members are extinct, at least two fundamental issues are intertwined: (1) what criteria should be used to assign a fossil to the Mollusca; and (2) what level of morphological distinctiveness is needed to establish a fossil as a representative of an extinct class. One may also ask how far the morphologic limits of extant classes should be extended. Several current textbooks recognize at least one class of extinct mollusks. There is no consistency in the number of extinct or extant classes in use, nor need there be while the field is still vigorously under discussion.

Key Words: classification, evolution, extinction, fossils, morphology

Class proposals for extinct mollusks, or fossils thought by some paleontologists to be mollusks, have been in the literature for about a century. Discovery of living examples of the Class Monoplacophora stirred both malacology and paleomalacology and the last four decades have seen new proposals of both extant and extinct classes. Study of specimens is far more informative than examination of photographs. Notwithstanding that point, sketches are included herein, but only for the purpose of making the text less obtuse. This essay repeats ideas written earlier (Yochelson, 1978, 1979) and, all my remarks should to be preceded with "in my view" or "in my opinion." Those fundamentally important phrases are eliminated, only to save space. Classification is a subjective activity and the higher one moves up in the Linnaean hierarchy, the greater the degree of subjectivity. "Authoritarianism is dangerous, especially for scientists, and the reader should approach my comments with a skeptical attitude" (Yochelson, 1979:324).

ISSUES

The more that has been learned of living animals, the more complex has become their classification. In 1758, Linnaeus did not use the category of phylum, but his equivalent has increased ten-fold. Over centuries, the relative importance assigned to various features by different workers has changed. For example, Molluscoidea was split off from Vermes and disappeared when Brachiopoda and Bryozoa came into use. The latter is now two phyla and some workers would also divide the former in two. The present concept of Mollusca is not necessarily sacrosanct. During one-quarter of a millennium, data on fossils have also dramatically increased and there is equally good reason to expect the number of higher-level taxa of extinct forms should also increase.

Defining a class as the taxon rank below a phylum is correct, though not particularly helpful. Much of acceptance or rejection in high-level systematics is based on the opinion of textbook authors. The fundamental question of whether extinct classes of mollusks are recognized is answered in the affirmative by reviewing paleontology texts of the last three decades. Consensus is that at least one extinct molluscan class, Rostroconchia (Pojeta *et al.*, 1972) is recognized in the Paleozoic; it features in a major paper (Waller, 1998). "One" is not large, but it constitutes a dramatic philosophical change from viewing Mollusca as containing only extant classes.

Which extinct class proposals are "reasonable" is another issue. The number of classes recognized among phyla varies widely. Within classes, the number of orders, families, or genera also varies widely. There are no rules to follow on the number and content of extinct and extant classes. How one decides whether a genus or a group of genera should be recognized as of class rank is yet a different issue.

A digression, which appears simplistic, is appropriate. The Class Gastropoda constitutes mollusks that have undergone torsion; all extant classes are based on features of soft anatomy. In the absence of torted soft parts, there are no fossils one can state with 100% confidence are Gastropoda. A paleontologist compares the shell of a living gastropod with a fossil shell; similarity of modern and fossil hard parts is at such a high level of probability, that the concept that it is a probability is forgotten. Classification is based on probability, not certainty.

For fossil mollusks to be considered an extinct class-rank taxon the hard part morphology should differ dramatically from that of other classes of Mollusca. To determine what features are dramatically different is as much a matter of art as it is of science. After defining species as a community of individuals "whose distinctive characters, are in the opinion of a competent specialist, sufficiently definite to entitle . . . a specific name" a fish specialist at the British Museum (Natural History), added "Groups of higher or lower rank . . . can be defined in the same way" (Regan, 1925:D1). In the game of classification, the only rule may be appeal to authority, a most unscientific approach. Cladistics and molecular systematics may appear more rigorous, but they too are ultimately grounded on certain assumptions; change the assumption and one changes the end result.

As a further complication in studying fossil mollusks that might have had more than one hard part, effects of taphonomy should not be ignored (Yochelson, 1984). Bizarre forms have been described, because modifications by sorting, transport, wear, and solution were ignored. Only after the taxon is satisfactorily defined by reference to the hard part(s) should one begin to speculate on soft parts and how they might have functioned. Listing interpretation of unknown anatomical features as the least significant criterion may not be popular, but it is the only way I see to understand molluscan fossils that do not closely resemble Recent mollusks. Three examples of possible extinct classes may help dispel some of this miasma. These are most familiar to me, but other proposals are equally worthy of discussion.

CLASS MATTHEVA

My proposal of this extinct class (Yochelson, 1966), which contained one genus, was based on the co-occurrence of two triangular-shaped, narrow pieces, each of which contained two deep cavities (Fig. 1). I was in error in suggesting the theoretical possibility of intermediate plates, for these were based on the presence of worn scraps. An assumption is that the cavities were for muscles and that two forms were anterior and posterior. I did not know which piece is anterior and which is posterior, and still do not know, but that uncertainly does not in any way affect the morphological uniqueness of this fossil. Specimens occur with algal domes. A paleoecological assumption is that the organisms did not cling to the substrate, but the weight of the two pieces helped maintain it in place during times the water was flowing.

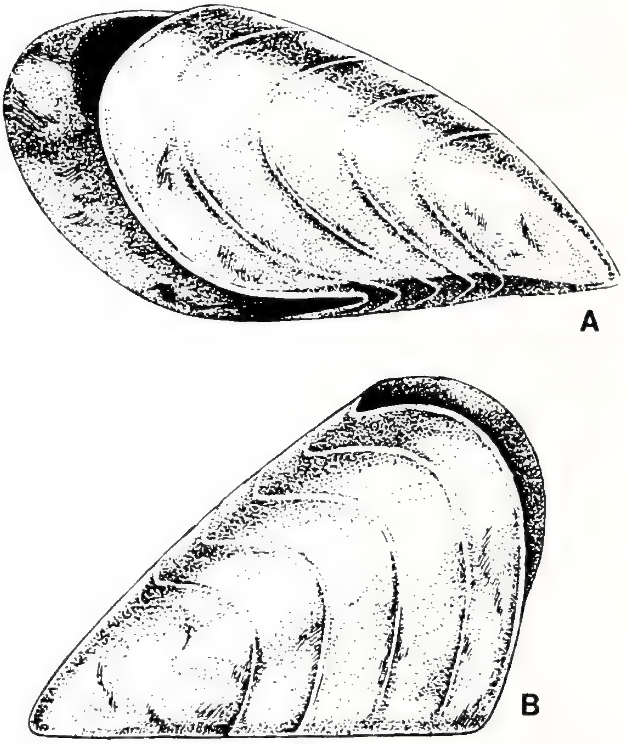

Fig. 1. Mattheva. **A.** One piece, possibly anterior. **B.** Another piece, possibly posterior. About three times natural size. (Yochelson, 1966, figs. 1 and 2).

Runnegar and Pojeta (1974) presented a reconstruction of the Late Cambrian *Matthevia*, and used a then undescribed intermediate (Runnegar *et al.*, 1979) to link it to an Early Ordovician polyplacophoran, *Chelodes*. That genus has only a single cavity and is broad and low, like the profile of a roof. I did not agree with their drawing, which resembled a seven-parted hedgehog (Yochelson 1978), nor did the latter suggestion of eight parts improve the reconstruction (Runnegar, 1983). There is evidence from fossils of only two thick heavy pieces, not the various slight modifications shown in their diagram. Runnegar *et al.* (1979) and Pojeta (1980) refuted my views, but it would be pedantry, to attempt to refute them here. Those not completely turned off by squabble should concordantly examine these papers.

One could expand the Polyplacophora to include *Matthevia*, strikingly different from all others, as is the approach in current literature. Alternatively, one could recognize a class that could have had more than one hard part, but whose parts do not resemble polyplacophoran plates of living taxa. My idea of a class has fallen on sterile ground, yet I remain mumpsimus. Because a paleontologist deals with hard parts, it is better to recognize smaller groups of more or less uniform "bauplan" than to have larger unwieldy groups. Gould (1989) used "shoehorn" for the process of cramming the wrong fossils into a taxon based on living material.

Polyplacophora have been reported from the Early Cambrian (Yu, 1984; 1990). If these fossils are part of the class – which I doubt – *Matthevia* is far off the main line of polyplacophoran evolution. If plates of *Preacanthochiton*, which most parties agree are polyplacophoran, are known from the earliest Early Ordovician, there is only a very short time to modify *Matthevia* from its bizarre shape to that approaching a modern chiton.

There are multiple possibilities in classification. A century and a quarter ago, von Ihering proposed that the Amphineura (= Polyplacophora of current literature) constituted a phylum. Most neomalacologists accept that polyplacophorans are quite different from most other mollusks and they are often put in a separate subphylum. Perhaps a phylum Amphineura, containing two classes, Polyplacophora and Mattheva would be appropriate. Of course, it does not follow that either von Ihering, Yochelson, or Runnegar *et al.* had the correct answer.

CLASS STENOTHECOIDA

My proposal for this class (Yochelson, 1968, 1969) was based on Early and Middle Cambrian bivalved fossils, commonly found as elongate, isolated valves; probably five or six genera fall within the class (Fig. 2). The valves are asymmetrical and inequivalved and have a single apical tooth and socket holding them together. Internally, one valve has many closely spaced ridges. Little can be mentioned about their paleoecology except that where they occur, they commonly are abundant. They are widespread in Asia (Yu, 1996) and North America.

As regards interpretation of *Stenothecoides*, "We offer the alternative suggestion that it may have been a bivalved monoplacophoran, with the lower (smaller?) valve formed by the sole of the foot" (Runnegar and Pojeta, 1974:316). This concept was repeated by Pojeta and Runnegar (1976:44), and seemingly there has been no further discussion.

Members of Class Bivalvia (= Pelecypoda of older literature) have two valves and if the choices were between that class or Monoplacophora, I would have preferred the former. There are inquivalve bivalves and asymmetric bivalves, but both are uncommon. The tooth and socket is quite unlike a hinge line. To place Stenothecoida within the Bivalvia would require such an expansion of the concept as to make it unpalatable. As an additional complication, a quarter of a century ago, the early Cambrian *Fordilla* (Pojeta *et al.*, 1973; Pojeta and Runnegar, 1974) was considered the ancestral member of Bivalvia. If that is true, then stenothecoids might be a dramatic morphologic expansion concurrently early in time with a conservative lineage. Adding this factor to the morphology, placing these aberrant fossils in an extinct class seemed reasonable.

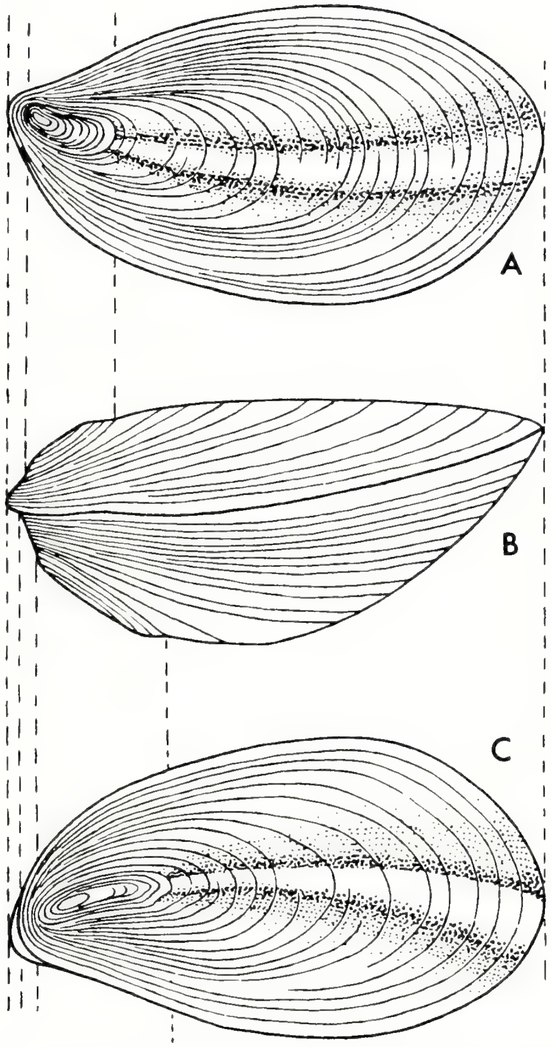

Fig. 2. Stenothecoida. A, dorsal, B, right side, C, ventral view of *Stenothecoides*. About ten times natural size. (Yochelson, 1969, fig. 3)

Although it is popular to speak of classification based on ancestor-descendent relationships, there is no way to determine these relationships apart from which comes first in the fossil record. An additional complication is that evolution need not proceed in a straight line. If one wanted to concentrate on diversity, an alternative could be to define

Mollusca as univalves. That done, a phylum Bivalvia might be proposed, with Stenothecoidea and Pelecypoda as classes within it. Bivalved gastropods are a complication, but one might make them another class of Bivalvia. At the least, this might stir debate on whether a classification based on hard part morphology of fossils should be subservient to one based on soft parts of living forms. Were such a phylum accepted, *Fordilla* could well form another class. My objections to its strange internal markings and my suggested reconstruction of soft parts different from typical Bivalvia has had no impact whatsoever (Yochelson, 1981)

"There is a significant stratigraphic gap in the fossil record of the pelecypods, between the occurrence of *Fordilla* in the late Early Cambrian and the Early Ordovician (Tremadocian-Arenigian) when the pelecypods undergo a major radiation" (Pojeta, 1975:371). Time gaps in the fossil record are to be expected where one is dealing with rare forms, but long gaps where the forms are widespread and abundant below and above a gap ought to suggest something is not quite right. In reporting a presumed pelecypod slightly older than *Fordilla*, Jell (1980:239) noted "Yochelson's other argument – the stratigraphic gap in the fossil record of pelecypods in Middle and Upper Cambrian – may disappear in the future as more finds are made." The gap is still there.

I do not really think that all bivalved mollusks will be placed in a separate phylum, but I mention this to show again that classification may be approached from different viewpoints. In turn that affects what is and what is not accepted as a class.

CLASS HYOLITHA

The hyoliths (Fig. 3) have been variously ancient "pteropods" (see Yochelson, 1979), a class of Mollusca (Marek and Yochelson, 1976), or a separate phylum (Runnegar *et al.*, 1975a). These fossils are abundant in some places in the Cambrian, but taper off rapidly in younger strata and die out by the end of the Paleozoic.

The most common hyolith order is the Hyolithida, bilaterally symmetrical closed tubes, uniformly expanding and generally with a triangular cross-section. The lower part of the aperture extends forward as a rounded shelf and the aperture is closed by an operculum; some opercula suggest a tooth and socket arrangement with the shell. Between the operculum and apertural margin are a pair of flattened curved pieces of calcium carbonate (= helens).

The Orthothecida are generally smaller, have a cross-section that may be circular, oval, or kidney bean-shaped among other configurations. The aperture lacks an anterior shelf. The operculum is simpler than that of the Hyolithida and does not show internal processes that would indicate hingement to the shell dorsum.

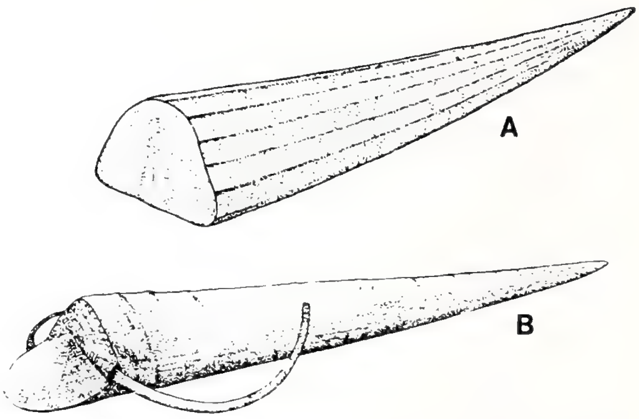

Fig. 3. Hyolitha. A. Reconstruction of *Orthotheca*. Slightly enlarged from natural size (Marek, 1963, fig. 13). B. Reconstruction of *Hyolithes*. Slightly reduced from natural size (Marek, 1963, fig. 12).

The Class Xenoconchia Shimanskiy (1963) contained Mississippian and Permian forms found in the Soviet Union. The older forms may have been platyceratid gastropods, which clung to crinoid calyxes and developed a variety of shapes, including nearly symmetrical cones. Some Permian fossils from Greenland may have been the largest Paleozoic fossil invertebrates, apart from giant cephalopods (Peel and Yochelson, 1981). In our judgement, these Permian xenoconchids were better placed as a third order within the Class Hyolitha (Peel and Yochelson, 1984). So far as I know the name Xenoconchia has not since appeared in the literature, except one suggestion that they are internal shells of cephalopods, an unlikely interpretation. These fossils have not entered into discussions on the class/phylum rank for the hyoliths.

The reconstruction of Runnegar *et al.* (1975) shows the closely folded gut of an orthothecid within the shell of a hyolithid. The peculiar "helens" projecting between operculum and apertural margin are fascinating, but they are an ordinal feature, neither a class characteristic nor a phylum characteristic. Others have objected to that reconstruction (Marek *et al.*, 1997).

However, "... it depends on one's concept of the phylum Mollusca. If one believes that all molluscs are descended from forms that had developed a dorsal exoskeleton, it is possible to exclude the Hyolitha from the phylum. The known muscle insertions of hyoliths suggest that their skeleton was not primitively dorsal" (Runnegar, 1978:332). Runnegar (1980) again discussed his concept of a phylum Hyolitha and, once they were removed from the Mollusca, they were not again discussed with that phylum

(Runnegar, 1983). Since the Monoplacophora are judged by Runnegar and others to be the stem group from which all other Mollusca are derived, the Hyolitha cannot be Mollusca. Possibly the only objective way to form an opinion of this highly subjective matter is for an interested person to read the paper by Runneger *et al.* (1975) coordinate with that of Marek and Yochelson (1976). Hyolitha are distinct from Gastropoda, or Rostroconchia, but I do not see that they are so vastly different as to be a separate phylum.

A tangential point is a recent redefinition of Monoplacophora in which that class term is abandoned for Tergomya (Peel, 1991). These forms, "classical" monoplacophorans if you will, begin in the Late Cambrian, not the Early Cambrian. Other small curved forms were placed in the extinct class Helcionelloida (Peel, 1991; Gubanov, 2000). It is too soon to claim that Helcionelloida has found acceptance as a molluscan extinct class, but its prospects are promising.

WHAT IS A PALEOZOIC MOLLUSK?

My involvement with Hyolitha brought the issue of how one defines a mollusk without considering soft parts. "Pragmatically defined a fossil mollusk is an organism whose hard parts show most of the following items: 1. The shell is composed predominately of calcium carbonate and may contain both calcite and aragonite; 2. The shell is layered and not pierced by holes; 3. The shell shows prominent growth lines; 4. The shell shows a logarithmic growth pattern; 5. The shell is basically a univalve, but may be modified to a bivalved condition; 6. The shell has basic bilateral symmetry, but may be modified to an asymmetrical condition; 7. The shell shows no trace of an apical attachment disk or foramen; 8. The shell may contain septa, either longitudinal or transverse; 9. The shell may have an operculum associated with it" (Yochelson, 1963:163).

That 1963 paper discussed the Class Coniconchia Liashenko, and I suggested two unrelated groups were involved, *Tentaculites* in a broad sense and *Hyolithes* in a broad sense. My objection was that Coniconchia seemed to have too wide a span of morphology, even though both forms were elongate tubes closed at the apex. Before anyone notes that I suggested both *Hyolithes* and *Tentaculites* might be mollusks, this was more than three decades ago and I knew even less than I know now. I have recanted on the latter. A considerable body of literature exists on both large and small tentaculitids. The small ones show more details of form and ornament than was anticipated half a century ago; possibly they were pelagic. Many of the large tubes are judged to have lived point down in sediment and presumably lived by filter feeding.

An elaborate systematic scheme has been developed for the Class Tentaculita (Farsan, 1994) which has been assigned to the Mollusca. My opinion is that including these fossils stretches the phylum beyond reasonable limits. They do not seem to have one of the basic features of Mollusca. The tube-like shell contains a multiple number of very thin layers; it is laminated. The Tentaculita are unique at the highest level and an extinct phylum is appropriate.

In a critique, "I proposed a list of features considered common to all or most molluscs (Yochelson 1961) [sic] in an attempt to define that phylum without reference to soft parts; there has been no discussion of this approach, nor of the features listed. The late Cambrian molluscan class Mattheva has no relationship to the various asymmetrical phosphatic sclerites discussed by Matthews and Missarzhevsky (1975:298-299): the latter separate these on morphological rather than chemical grounds. It does not follow, however, that all fossils with hard parts of calcium carbonate are molluscs, and there is no consensus among workers as to what fossils should be included in the phylum or excluded from it. Thus, Runnegar *et al.* (1975) suggest that *Hyolithes* and its allies constitute an extinct phylum, whereas Marek and Yochelson (1976) continue to place them as an extinct class of Mollusca" (Yochelson, 1975). There still has been essentially no more discussion of characterizing Mollusca from only the hard parts.

It is not easy to define a mollusk, living or dead. "Because no single soft- or hard part character or combination of a relatively few characters, is common to all mollusks, it is not possible to frame a succinct morphological definition of the phylum Mollusca as can be done for such phyla as the Echinodermata and Chordata. ... Mollusks are unified by morphological gradations between the different forms, by embryological similarities, and by information deduced from fossils of the various classes assigned to the phylum" (Pojeta, 1980:55).

It seems to me that possession of the mineral aragonite is basic to Mollusca and could be the reason that many fossils are not well preserved; reversion of the original shell to calcite may explain why most shells preserved in limestone exfoliate and leave only the internal mold. Immediately following in importance to shell mineralogy, I would list shell structure. A "thought experiment" is to imagine that the scaphopods are all extinct and wonder how they would be classified. Probably they would be considered "worm tubes" until someone making thin-sections saw a similarity to the shell structure of gastropods.

Similarly, one could imagine that Cephalopoda were known only from the living octopus in today's seas. Where would paleontologists place all the straight, curved and coiled septate fossil shells. If they were satisfied by shell composition, and microstructure that these were mollusks, surely the fossils would have to be assigned to a class, all of whose members were extinct. Debate between malacologists and paleontologists could break down as each group looks at different features for high-level classification within the Mollusca.

Worm tubes can produce an amazing variety of shapes and it might be educational and enlightening to compare the presumed Ordovician and Silurian larval gastropods of Dzik (1994) with a Middle Ordovician population of highly variable worm tubes (Bockelie and Yochelson, 1979). Every author, including the present one, is selective in citation of references. For an excellent example of this process, Dzik illustrated *Januspira* as a monoplacophoran, following Runnegar (1977), whereas an interpretation on the following page (Yochelson, 1977) of it as a bizarre worm tube was ignored.

In the past, a number of strange fossils were tossed into the Mollusca. A Paleozoic scaphopod may be a worm tube (Yochelson and Goodison, 1999) or a monoplacophoran may be a medusoid (Webers and Yochelson, 1999); it depends on what features one considers to be most significant. To begin to make sense of the Mollusca, one should remove those fossils that ought not to be in the phylum. Unfortunately, by some workers ignoring composition of the integument, or great variation in shape, or concentrating on internal molds, there is danger of further confusing the fossil record of the Mollusca.

EVOLUTION

Patterns in evolution is another subject with much discussion and little resolution. One notion that may have wide acceptance is that of adaptive radiation - a new form appears and diversifies rapidly, at least rapidly on the geologic time scale. The concept was first clearly enunciated in regard to early Cenozoic land mammals, but it seems to apply to other organisms, plant and animal, at other times and at various taxonomic levels. There is a near consensus that the early record of both Arthropoda and Echinodermata includes much high-level differentiation; in the Echinodermata, the number of extinct classes could be double those that are extant (Campbell and Marshall, 1987).

If adaptive radiation is a general phenomenon, why does the phylum Mollusca have a different pattern? In one evolutionary scheme (Runnegar and Pojeta, 1974; Pojeta and Runnegar, 1976) there is room for only one extinct class. "At least six [of the eight] higher taxa of Mollusca originated in the Early Cambrian and did not begin to radiate in any substantial way until the late Cambrian or early Ordovician" (Runnegar, 1987:50). In this scheme, the Cephalopoda is the only class that appears in the Late Cambrian and immediately undergoes radiation. Scaphopoda appeared even later in the fossil record.

In an alternative interpretation (Yochelson, 1978) most Cambrian mollusks superficially resemble those in extant classes, but are actually products of an early adaptive radiation; the extant classes come later. It is impossible to demonstrate that one of these two approaches is true and one is false, but I would be less than human if I did not prefer my view. With the latter approach one will seek and find fossils that belong to extinct classes, and with the former, one will not. Fossils do not have their systematic position inscribed on the shell and there is room for honest disagreement when unusual or poorly preserved material is at hand. Despite what has been written (Runnegar and Pojeta, 1974), there is no single paleontological viewpoint.

I am not qualified to discuss the mechanism of evolution of classes. In 1978, I guessed at a non-shelled form beginning in the Precambrian and existing to at least mid-Paleozoic as the source for the various classes, but now think it was a bad guess. Changes that are recognized as being of class rank could have developed in the larval stage. I do not necessarily think that major steps happened instantly, but with the current resolution in stratigraphy, they appear to be instantaneous.

The similarity of shell throughout the Mollusca may suggest that an organic template formed originally and then the mollusks diversified. The mechanism of formation of hard parts in the Phanerozoic is still a murky area. It can be made murkier, for did the hypothetical noncalcified mollusk form a calcified hard part or parts, or did having a platform on which to anchor musculature result in a fundamentally different organism? Put more starkly, did the mollusk make a shell or did the shell make the mollusk?

SUMMARY

"The poor Middle and Late Cambrian record of the Gastropoda, Pelecypoda and Rostroconchia . . . is difficult to explain. Possibly more fossils of these groups will be found when more microfossils are extracted from Cambrian rocks" (Runnegar, 1978:333). We are still waiting. If gaps in the record are real, there may be merit in considering that the later record of the mollusks consists of convergent forms rather than direct ancestors.

As mentioned, a fundamental problem is what fossils should be placed in the Mollusca. A second problem is what is the level of distinctiveness that makes one fossil a representative of an extant class and another a representative of an extinct one. Even if those points are resolved, others may be irreconcilable. In his view, Runnegar (1983) refuted my refutation of the Runnegar and Pojeta hypothesis. This "yes it is and no it is not" has hardened both positions. If one side were to concede that hyoliths were not mollusks, would the other concede that the rostroconchs are simply peculiar pelecypods? In the long run, science might not be better served, but we could save a lot of time and paper if we agree that there are no extinct classes of mollusks.

For some specialists, "Higher taxa are recognized largely by hindsight, after sufficient evolution and diversification have produced a cohesive group of related organisms" (Runnegar, 1978:329). Likewise "If this symposium had been held in the Early Cambrian, it is probable that *Fordilla* and *Pojetia* would, at best, be ranked as a family or superfamily of the Monoplacophora" (Runnegar, 1987:49).

It is a cheap shot to note the clarity of 20/20 hindsight vision. In investigations of classification, should emphasis be on the product – morphology of the organism itself – or on the process – interpretation of ancestor-descendent relationships? My position is that one classifies on the basis of the features that are preserved. I admit this is very much an old-fashioned viewpoint.

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AMERICAN MALACOLOGICAL SOCIETY, INC.
FINANCIAL REPORT

General Accounts

1999 Income and Expenses

TOTAL ASSETS (1/1/99)			$174,042.52
INCOME			$ 30,440.69
Membership Dues (1996, 1997, 1998)		1,962.00	
Membership Dues (1999)		12,047.00	
Membership Dues (2000)		15.00	
Interest and Dividends		5,080.46	
Money Market Account Interest	1,132.73		
Life Membership Endowment Fund	345.84		
Symposium and Student Grant Endowment Funds	3,601.89		
Publications Income		4,547.15	
AMB Foreign Subscriptions	1,372.00		
AMB Domestic Subscriptions	2,565.00		
AMB Page Charges	296.00		
AMB Back Issues	10.15		
AMB Reprint Charges	160.00		
Sales of Other Publications	144.00		
Annual Meetings		2,248.50	
1999 Auction Proceeds	2,248.50		
Donations		4,520.00	
Symposium Endowment Fund	20.00		
Student Grant Endowment Fund	1,400.00		
Bernice Barbour Foundation	3,000.00		
For Reprinting Revised "How To Collect"	100.00		
Miscellaneous Income		20.58	
EXPENSES			$27,791.16
Treasurer Expenses		1,293.11	
Affiliate Memberships		135.00	
Banking Fees		27.51	
MC - VISA Fees		725.85	
Incorporation Fee		40.00	
Insurance/Bond Fees		605.00	
Managing and Bulletin Editor Expenses		626.14	
Publication Costs		13,872.20	
AMB	11,237.08		
AMS Newsletter	932.12		
Brochures	608.00		
Directories	1,095.00		
Dissolving Council of Systematic Malacologists		52.00	
Student Research Grants		2,000.00	
1998 Abstracts Mialing		137.36	
1999 Meeting Expenses - Separate Account			
Travel Expenses (To Meetings)		3,776.99	
Bulletin Editor (1999)	1,487.33		
Managing Editor (1999)	294.40		
Treasurer (1999)	1,995.26		
Student Paper Awards		1,500.00	
Bernice Barbour Student Travel Awards		3,000.00	
NET GAIN IN 1999			$2,649.53
TOTAL ASSETS (12/31/99)**			$179,908.98

**Includes an increase in capital investments. The above income and expenses do not include the annual meeting accounts.

67th ANNUAL MEETING
THE AMERICAN MALACOLOGICAL SOCIETY
WORLD CONGRESS OF MALACOLOGY
VIENNA, AUSTRIA
AUGUST 19 - 25, 2001

The American Malacological Society will be meeting jointly with Unitas Malacologica in Vienna, Austria, from 19-25 August 2001, at the second World Congress of Malacology. Scientific presentations will take place in the lecture rooms of the "Biozentrum" of the University of Vienna (founded in 1365!). A variety of housing facilities from student dorms to hotels will be available, all within 20 minutes of the meeting venue.

Five symposia are currently being organized:

- Evolution and Development in Molluscs (organizers Gerhard Haszprunar and W. A. G. Dictus)
- Chemosymbiosis (organizers Carole Hickman, Penelope Barnes, and Martin Zuschin)
- Mollusca in Long-lived Lakes (organizers Frank Wesselingh and Elinor Michel)
- Molluscan Conversation (organizers Ian Killeen and Mary Seddon)
- Functional Morphology (organizers Dianna Padilla and Shirley Baker)

English is the preferred language of scientific presentations. Oral contributions will be limited to 15 minutes, including discussion; poster presentations are strongly encouraged, as is the organization of workshops on special topics. There will be two poster sessions.

Field trips to Schneeberg (alpine gastropods), the Danube alluvial forest (limnic and terrestrial molluscs), and to the tethyan and paratethyan fossil sites will provide a break in the scientific program.

A Curator Session at the Naturhistorisches Museum Wien will be organized by Helmut Sattman and Anita Eschner.

The Austrian capital offers a sparkling array of scientific, historical, architectural, and musical highlights, both within its borders and in its nearby surroundings. The social program will include a Congress Opening Reception at the Naturhistorisches Museum Wien and a Congress Dinner at the Rathaus (City Hall) under the patronage of the mayor of Vienna, Dr. M. Häupl, who is a zoologist (!). In addition, a mid-week dinner at a "Heurigen" (typical Viennese open-air wine tavern) is planned. There will also be the traditional AMS auction led by Dick Petit.

AMS will be providing up to six $500 travel awards for graduate students to attend the meeting. These awards will require a special early application and will be available on a competitive basis. Details will be posted on th AMS website (http://erato.acnatsci.org/ams/), the Mollusca listserver, and in the spring newsletter of the American Malacological Society.

Preliminary registration is available at the meeting website:
http://www.univie.ac.at/WCM2001/index.htm.

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IN MEMORIAM

Ruth D. Turner

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PRIMARY MOLLUSCAN TAXA INDEX

[first occurrence in each paper recorded; new tax (including species) in **bold face**]

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CONTRIBUTOR INFORMATION

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Hillis, D. M. 1989. Genetic consequences of partial self fertilization on populations of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). *American Malacological Bulletin* 7(1):7-12.

Seed, R. 1980. Shell growth and form in the Bivalvia. *In: Skeletal Growth of Aquatic Organisms,* D. C. Rhoads and R. A. Lutz, eds. pp. 23-67. Plenum Press, New York.

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